

Universidad de Huelva

**Departamento de Ingeniería Química, Química Física y
Ciencias de los Materiales**



Optimización de la formulación y procesado de emulsiones aceite/agua de interés nutricional estabilizadas con fosfolípidos de distintos orígenes

**Memoria para optar al grado de doctor
presentada por:**

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Fecha de lectura: 15 de septiembre de 2017

Bajo la dirección de los doctores:

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Huelva, 2017



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Investigation field for dissertation: Procesos y Productos Químicos

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Authorship Attribution

The undersigned hereby declares to be the core author of this thesis, whereby, this systematically developed and categorically derived work has been reported entirely under the sole supervisions of Prof^a. Dr^a. Maria Carmen Sánchez Carrillo and Prof. Dr. Crispulo Gallegos Montes, from Chemical Engineering department UHU- Huelva and I&DC Complex Formulations department Fresenius Kabi Deutschland GmbH, respectively.

It is also confirmed that where the thesis is based on consulted/quoted published papers, or where a joint work was performed, a clear and named references were made as of bibliography of what the contributions of others were.

(Muhammad Anjum Nawaz)

Acknowledgements

The author of this dissertation would cordially like to admit that virtually it is almost impossible to undertake a study of any magnitude without the cooperation and assistance of people who are generous not only with their time, but who make the conscious decision of laying on the line deeply personal vestiges of themselves. I pay my sincere gratitude to my loving university professors; Prof Dr. Jose Maria Franco Gomez, Prof Dr. Crispulo Gallegos Montes and Prof^a Dr^a. Concepcion Valencia Barragan, who, despite exacting schedules, availed themselves as critical readers or casual commentators at various stages during the research process. Their input carved in shape for this research thesis in significant ways. I am highly obliged with their tremendous efforts and time to time guidance along the long run.

I am also sincerely grateful to the generous financial support I received from Fresenius Kabi Deutschland during the entire period of my research work here at Universidad de Huelva and my hiring as an intern there at the facility of Fresenius Kabi, Bad Homburg Germany. Without this aid, it is out of question that I was not able to proceed with the said study. This aid includes not only financial terms but procurement of raw materials, equipment usage in the line of experimental/characterization work and above all an expertise opinion from Industry always having a practical significance. I pay my sincere regards to Dr^a. Lida Quinchia Bustamante and Mr. Getachew Assegehegn from Fresenius Kabi who were of great help during all this process. The prompt and professional processing of my requests from the Fresenius Labs, meticulously by them, paved my path in process optimization and product handling during characterization and stability protocols.

I would like to place on record my heartfelt thanks to my supervisor, Prof^a. Dr^a. M^a Carmen Sánchez Carrillo, for reading with refreshing insight, and fine-grained critical astuteness of the various drafts of the thesis. Her sophisticated theoretical mind and contempt for mediocrity has been instructive and inspiring. I am mostly thankful that she afforded me the autonomy and latitude to think my own thoughts, and follow my scholarly instincts, by always encouraging me and giving me an open hand.

I am truly privileged by one of my best colleagues and friends to be a great support during the dissertation work, Dr. Francisco Ortega Bravo, who was touched with rare sincerity. I have often marveled at the blessing of having him during the hard times in writing dissertation. I take this opportunity to literally thank him for believing in me and pointing me in the direction of important resources, and for taking such a keen interest in my work and well-being.

To my friends both near and far; to those I love and those I passed my days and nights, thanks for the encouragement, for listening patiently, politely and with palpable enthusiasm while I perfected the art of complaint about the woes and worries of doctoral studies. Special thanks to my dear friends Waqas A. Bajwa for always helping me and sharing with me toughest times, Usman Hameed and Muhammad Ayub for the far company and keeping alive the true spirits of friendship composed in the liminal space of virtual reality, for their spirit of brute adventure and determination, I nestled them all in the deep recesses of my mind during much of the writing process.

Finally, my endearing and enduring gratitude goes to my family: mom R. Sultana, father M. Nawaz, wife S. Kousar and my two kids, Afnan and Samad, who have remained the bedrock of my scholarly and personal (mis)ad/ventures. Ironically, at the end of a study that has relied on narrative research to tell personal and professional stories, I'm left with a contented smile and hereby thank my Lord- Almighty Allah, to keep me embraced and fulfill this task.

This dissertation is dedicated to my family and friends whom never ending support, help and encouragements enabled me in a step wise completion of this work in the long run.

Also, I would like to dedicate this piece of work to my country and especially to the Higher Education Commission, Pakistan- who allowed me to fulfill the task in extended time period

Summary

Developing a new pharmaceutical product in the form of a parenteral emulsion, that being the target of this research work, involves not only characteristic properties of the product formulation scientifically justified but also abiding by the regulations from the conforming authorities, apart. Parenteral formulations, as the manufacturing scope of this thesis, are used not only as pharmaceutical products but also have nutritional values too, whereby using intravenously, these emulsion formulations are integral part of total parenteral nutrition (TPN). Constituents and characteristic properties of these formulations, in this way, follows far more restrict protocol of conformance in their production and clinical usage than other nutritional products.

The use of lecithin, as a food based product, in the form of emulsifier in parenteral emulsions has long been recognized and many commercial parenteral formulations consume lecithin of egg and soy origin in their parenteral products. The adverse events related to autopsy findings that majorly include intravascular fat accumulation were the cause for finding sources of fats other than soy oil and emulsifiers other than commercially used lecithin with egg origin (Intralipid®). In this thesis, commercially available lecithin with different origin was used as an emulsifier to qualify emulsions as a parenteral product initially utilizing different oil types in the formulations. Olive oil, soy oil and fish oils were used as fat source in these formulations at pH values ranging from 2 to pH 9. Different pH controlled emulsions were produced with lecithin from soy, sunflower and rapeseed origin and a stability profile was generated on the basis of creaming evaluation, PSD and zeta potential characterization. Results indicated that the parenteral formulations with pH values higher than 7 were the most stable in this scenario with different oil types and lecithin origin. Collectively, concentrated fish oil emulsions have the least stability profile even at higher pH values. Soy oil emulsions gave maximum stability duration at pH 8 and 9 with different lecithin origin at commercial concentrations as an emulsifier i.e. 1.2%.

The knowledge of these stability profiles at different pH values for different oil types and emulsifier concentration was utilized in order to develop novel emulsifier systems for parenteral emulsions. In this research work, formulations containing new emulsifier systems with marine origin, FKKO42 and FKHEO30, were developed at only higher pH values with different emulsifier concentrations (i.e. 0.6 to 2.4%). These new emulsifiers have dual properties fulfilling not only nutritional demands but having emulsification characteristics as well in a parenteral product. The PSD and zeta potential profile of FKKO42, containing sufficiently high

phospholipids, equal to 42%, at a concentration of 1.8 and 2.4 gave stable and repeatable emulsions within pharmacopeia tolerance limitation ($PSD < 0.5 \mu m$ and $\zeta > \pm 30 mV$).

The shelf life of FKKO42 was sufficiently long enough with respect to initial studies and even at higher temperatures (40 °C) for accelerated stability studies, the particle size did not change and emulsions were stable throughout their stability period. The results of 1.8 and 2.4% FKKO42 were almost similar for all oil types used (soy, fish, omegaven) while for another fat source used in this study comprised of a mixture of oils collectively known as SMOF (soy, medium chain triglycerides (MCT), olive and fish oil) with 20% oil loadings, due to higher oil contents of the emulsions, a 2.4% emulsifier quantity was preferable in order to keep the emulsions stable at accelerated stability study. Emulsions with 0.6% FKKO42 emulsifier were marked unstable as free oil drops were found at the top after formulation processing. Lab preparations of these emulsions containing FKKO42 produced a characteristic top emulsifier layer containing no naked oil drops at the said pH (8-9) and were confirmed through microscopic studies as well. The pilot plant production of the same overcame that characteristic layer during processing with no significant alteration in overall physical properties of the formulations containing FKKO42.

Similarly, formulations with FKHEO30 emulsifier in concentrations with 1.8 and 2.4% were extremely unstable and their water/oil interphase separated out after preparations. The emulsifier concentration was reduced down to 0.6, 0.9 and 1.2% to study FKHEO30 emulsifier formulations stability. It was observed that at least formulations were possible with lower concentration of FKHEO30 emulsifier; however, there was clear evidence of free oil drops at the top of each formulation and confirmed through microscopic studies too. The stability study was continued though; no satisfactory results were predicted by using FKHEO30 emulsifier in the parenteral emulsions.

FKKO42 emulsifier with 1.2 to 2.4% concentrations in the formulations at pH values between 8 and 9 is potentially a new emulsifier for the development of new parenteral emulsions comprising dual properties of marine nutrition in the form of omega-3 fatty acids, as well as processing aid in emulsification. Consequently, commercially available lecithin emulsifiers could be replaced entirely with all their complications based on long term dosage for any parenteral intravenous (IV) product with this novel emulsifier. Characteristically, FKKO42 emulsions seemed appropriate candidate for the future of parenteral emulsions abiding by regulations from USP (United States Pharmacopeia) and Eur-Pharmacopeia (European Pharmacopeia).

Resume

Desarrollar un nuevo producto farmacéutico en forma de emulsión parenteral es el objetivo de este trabajo de investigación que implica no sólo propiedades características de la formulación del producto científicamente justificadas, sino también el cumplimiento de las regulaciones de las autoridades conformadoras, competente. Las formulaciones parenterales, se usan no sólo como productos farmacéuticos sino que también tienen valores nutricionales, por lo que son parte integral de la nutrición parenteral total (TPN). Los constituyentes y propiedades características de estas formulaciones, de este modo, siguen un protocolo mucho más restringido en relación a su producción y uso clínico que otros productos nutricionales.

El uso de lecitina, como producto alimenticio, en forma de emulsionante para emulsiones parenterales viene siendo habitual, ha sido reconocido desde mucho tiempo y muchas formulaciones parenterales comerciales incluyen la lecitina de huevo y soja en sus productos parenterales. Los eventos adversos, que incluyen principalmente la acumulación de grasa intravascular, fueron la causa para buscar fuentes de grasas distintas del aceite de soja y emulsionantes distintos de la lecitina de origen animal (Intralipid®). En esta tesis, se utilizó lecitina disponible comercialmente con diferentes orígenes como emulsionante con el que se pudo preparar emulsiones parenterales es que inicialmente utilizaban diferentes tipos de aceite en las formulaciones. Se utilizaron aceite de oliva, aceite de soja y aceites de pescado como fuentes de grasa en estas formulaciones, a pH entre 2 y 9. Se produjeron diferentes emulsiones con control de pH con lecitina de origen de soja, girasol y colza y se generó un perfil de estabilidad sobre la base de la evaluación del cremado, distribución de tamaño de gota (PSD) y potencial zeta. Los resultados indicaron que las formulaciones parenterales con valores de pH superiores a 7 fueron las más estables con diferentes tipos de aceite y diferente origen de las lecitinas. Colectivamente, las emulsiones de aceite de pescado concentrado (omegaven) tienen el perfil de menor estabilidad incluso a valores de pH más altos. Las emulsiones de aceite de soja dieron una duración de estabilidad máxima a pH 8 y 9 con lecitinas de orígenes diferentes a concentraciones comerciales de emulsionante, es decir 1,2%.

El conocimiento de estos perfiles de estabilidad a diferentes valores de pH para diferentes tipos de aceite y concentración de emulsionante se utilizó con el fin de desarrollar nuevos sistemas emulsionantes para emulsiones parenterales. En este trabajo de investigación, las formulaciones

que contenían nuevos sistemas emulsionantes de origen marino, FKKO42 y FKHEO30, se preparan sólo a valores de pH más altos con diferentes concentraciones de emulsionante (de 0,6 a 2,4%). Estos nuevos emulsionantes tienen propiedades que cumplen no sólo las exigencias nutricionales sino que tienen características emulsificantes también en un producto parenteral. El perfil de PSD y potencial zeta de FKKO42, con contenidos de fosfolípidos suficientemente altos, igual al 42%, a una concentración de 1,8 y 2,4 dieron emulsiones estables y repetibles dentro de la limitación de tolerancia a la farmacopea ($PSD < 0,5 \mu m$ y $\zeta \geq \pm 30 mV$).

La vida útil de FKKO42 fue suficientemente larga con respecto a los estudios iniciales e incluso, a temperaturas más altas (40 °C) para estudios de estabilidad acelerada, el tamaño de gota nucleó no cambió y las emulsiones fueron estables a lo largo de su período de estabilidad. Los resultados obtenidos con 1,8 y 2,4% de FKKO42 fueron casi similares para todos los tipos de aceite utilizados (soja, pescado, omegaven), mientras que para otra fuente de grasa utilizada en este estudio se compuso una mezcla de aceites conocidos colectivamente como SMOF (soja, triglicéridos de cadena media, aceite de oliva y aceite de pescado) al 20% de carga de aceite. Debido al mayor contenido en aceite de las emulsiones, se prefirió una cantidad de emulsionante al 2,4% para mantener las emulsiones estables en el estudio de estabilidad acelerada. Las emulsiones con emulsionante FKKO42 al 0,6% se marcaron como inestables ya que se encontraron gotas de aceite libres en la parte superior después del procesamiento de la formulación. Las emulsiones que contenían FKKO42 dieron lugar a una capa de emulsionante superior característica que no contenía gotas de aceite libre a dicho pH (8-9). Se confirmaron también mediante estudios microscópicos. La producción en planta piloto de la misma emulsión superó esa capa característica durante el procesamiento sin alteración significativa en las propiedades físicas globales de las formulaciones que contenían FKKO42.

El emulsionante FKKO42, con concentraciones de 1,2 a 2,4% en las formulaciones, a valores de pH entre 8 y 9 puede ser potencialmente un nuevo emulsionante para el desarrollo de nuevas emulsiones parenterales que comprendan características de nutricionales, en forma de ácidos grasos omega-3, y co-ayudante en la emulsificación. En consecuencia, los emulsionantes de lecitina comercialmente disponibles podrían reemplazarse completamente con este nuevo emulsionante. Característicamente, las emulsiones de FKKO42 parecen candidatas apropiadas para el futuro de las emulsiones parenterales respetando las regulaciones de USP (Farmacopea de Estado Unidos) y Eur-farmacopea (Farmacopea de Europeo).

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List of Abbreviations

α -LA (ALA)	α - Linolenic Acid
CO ₂	Carbon dioxide gas
DAA manual	Dietitians Association of Australia
DHA	Docosahexaenoic Acid
DI water	De-Ionized Water
DLVO	Derjaguin, Landau, Verwey, Overbeek theory
EFA D	Essential Fatty Acid Deficiency
EPA	Eicosapentaenoic Acid
EUR Pharmacopeia	European Pharmacopeia
FA	Fatty Acids
FFA	Free Fatty Acids
FKHEO30	
FKKO42	
GmbH	Limited company
GMP	Good Manufacturing Practice
GRASS	Generally Recognized as Safe Substances
HCl	Hydrochloric Acid
HLB	Hydrophilic, Lipophilic Balance
Homo	Homogenization
IEP	Iso Electric Point
IFALD	Intestinal Failure Associated Liver Disease
INS	Infusion Nursing Society
IV	Intravenous
IVFE	Intravenous Fat Emulsions
LA	Linoleic Acid
LPC	Lyso- Phosphatidylcholine
LPE	Lyso - Phosphatidylethanolamine

MCT	Medium Chain Triglycerides
N ₂	Nitrogen Gas
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NO OL (NOL)	No (sodium) oleate
O/W	Oil Water Emulsions
O/W/O	Oil, Water and Oil emulsions
OA	Oleic Acid
PA	Phosphatidic Acid
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PEG	Poly Ethylene Glycol
PFAT5	Percentage of Fats less than 5
pH	Power of Hydrogen Ions concentration in a solution
PL90	Phospholipid - 90
PN	Parenteral Nutrition
PSD	Particle Size Distribution
PUFAs	Poly Unsaturated Fatty Acids
R LPC	Rapeseed Lyso- Phosphatidylcholine
SMOF	Soy, MCT, Olive and fish oil mixture
SO	Sodium Oleate
TDS	Technical Data Sheet
TPN	Total Parenteral Nutrition
UHU	Universidad de Huelva
US FDA	United States Food and Drug Association
USP	United States Pharmacopeia
W/O/W	Water, Oil and Water emulsions
WFI	Water For Injection

List of Symbols

%	Percentage
a	Absolute values (modulus)
μm	Micro meter
D _(4,3)	Volumetric diameter
g/cm^3	Grams per centimeter cubic
gms	Grams
h	Hours
H ⁺ /OH ⁻	Hydrogen positive and Hydroxyl negative ions
L	Liter
mins	Minutes
mOsmol/kg	Milli osmole per kilogram
mS/cm	Milli siemens per centimeter
mV	Milli volts
N	Normality
Na/K buffer	Sodium or potassium salt buffers
nm	Nano meter
°C	Degrees Celsius
psi	Pounds per square inch
rpm	Revolution per minute
®	Trade mark
w/w	Weight: weight
ω -3	Omega -3
ω -6	Omega -6
ω -9	Omega -9
ζ - potential	Zeta potential

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Synopsis

Current thesis entitled “*Optimización de la formulación y procesado de emulsiones aceite/agua de interés nutricional estabilizadas con fosfolípidos de distintos orígenes*” is an outcome of a collaboration project between Universidad de Huelva and Fresenius Kabi Deutschland GmbH. The aim of this study is to develop progressive pharmaceutical products using novel emulsifiers in the form of parenteral formulations with a drug importance of worth lifesaving. The approach utilized in this dissertation is practically an industrial approach with an outcome of commercially viable pharmaceutical formulations.

The rationale behind this research was to find out new phospholipid systems to develop and optimize the formulations in the form of oil-water (o/w) emulsions that have nutritional values. To this extent, development and formulation of various kinds of o/w emulsions using distinct phospholipids as emulsifier with different origins was the scope of this research work.

This thesis is divided in three parts while keeping preamble of the main strategy on these parts mainly. In the first part, a brief introduction on formulation's constituents and the characteristics of intravenous emulsions was discussed. Reference was made to the characterization sequence and the motivation behind the characterization with respect to the pharma standards as explained by the regulatory authorities (United States Pharmacopoeia, USP, etc.)

The second part explains the subject of stability of o/w emulsions stabilized by commercially available different plant and egg origin phospholipids, mainly lecithin, a food based amphiphilic phospholipid. Various factors for stability and characterizing these emulsions on the basis of stability criteria established in part I are discussed. The knowledge acquired by vast variety of formulations prepared and characterized with respect to vegetable and egg origin lecithin at a series of pH values, formed the basis for the subsequent parts of this thesis, where the main concern was to optimize the processing conditions in order to improve the stability of o/w emulsions containing novel phospholipids with distinct origin i.e. animal and/or marine phospholipids as emulsifiers.

Third part of this thesis starts discussion on o/w emulsions for parenteral nutrition (PN) formulated precisely at higher pH values only (i.e. pH 8-9 units). Novel emulsifier system with marine origin phospholipids termed as FKHEO30 and FKKO42 were used distinctly to prepare formulations and characterized with different concentration of these marine origin emulsifiers at

typical parenteral emulsion's pH values indicated above. Various oils types as fat source were used to formulate using these emulsifiers and results were compared to the existing commercial products in the market with the same oil/fat source.

Earlier, in third part, stability criterion for FKHEO30 marine phospholipid was set along with a series of emulsifier concentration for the stabilization of parenteral emulsions. The stability threshold results obtained from this type of marine phospholipid have described another series of emulsion formulations based the 2nd type of marine phospholipid (i.e. FKKO42) and would be dealt later in this section separately. Lateral section of part three of this thesis consists of discussion on results obtained after using this new marine based emulsifier (marine phospholipid- FKKO42) whereby concentrating the focus on the stability of these novel formulations using marine origin emulsifier.

The optimization of the concentration of emulsifier to formulate an emulsion with its prolonged shelf/stable life was the main objective of introducing a novel emulsifier. The best concentration within parenteral pH values allowance, fulfilling stability criteria is discussed in this section along with a series of different emulsions formulated at different concentrations and choosing arguments on the optimization of the emulsifier for parenteral nutrition

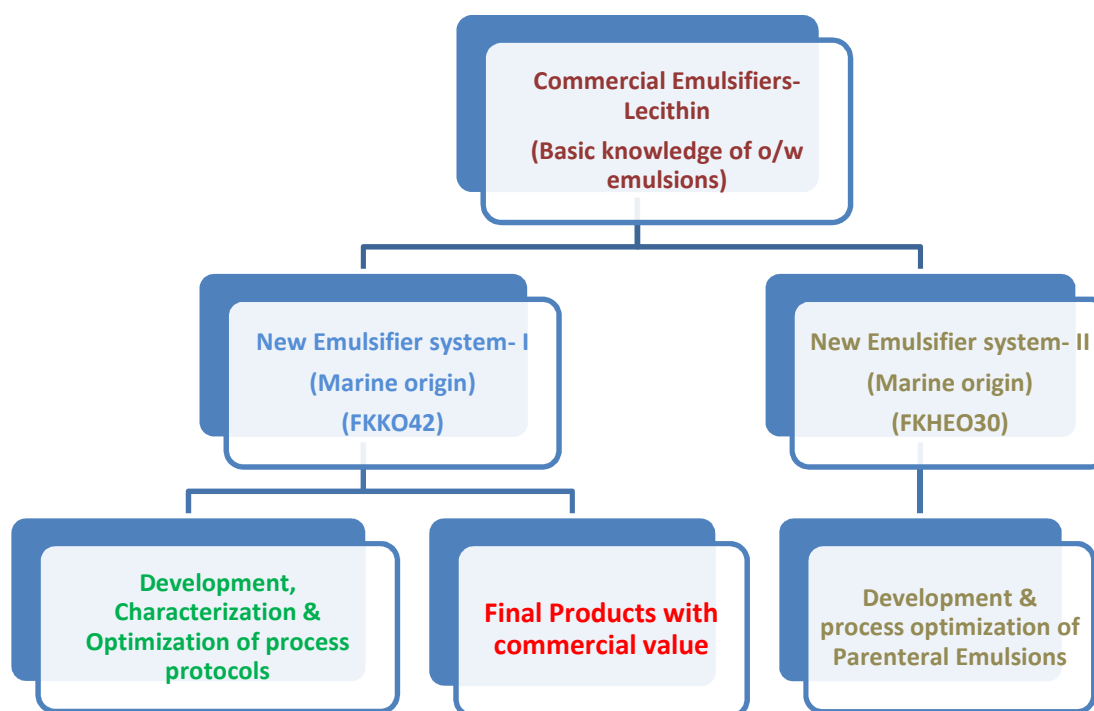


Figure1: Flow Chart for the structural organization of thesis

The state-of-the-art processing and characterizing techniques used within this research work have been only possible along with continuous collaboration from Fresenius Kabi Deutschland GmbH.

Part I:

Formulation Constituents and Product Technology: An Overview

1. Formulations: An Introduction

Fundamentally, formulation is putting together components in appropriate relationships or structures, according to a formula. In pharmaceutical sciences, It is the process in which different chemical substances, termed as active chemical substances, are combined together to produce a medical compound i.e., a drug. (Hassan, 2012). Formulations can be a solid, semisolid or a liquid origin depending upon applications.

The essential constituents of a formulation basically are: a pharmaceutically active drug and a carrier to take it. Now, depending upon the routes of administration, the target application site and the characteristic formulation properties, the formulations are considered simple or complex. Other than a simple carrier/drug duo, the virtue of a complex formulation not only comes from the fluid complex rheological properties defining constituent formulae, but also from their formulation technique of manufacture and route of administration too, with a specified targeted delivery for certain applications (Linda, 2013).

Specified route of administration and formulation techniques are the major concerns of this project. So, here in this dissertation, main emphasis is given on the formulations for their targeted application while considering the routes of administration in regard to the formulation technology. Before technological stand point of complex formulations might be discussed in detail in this part of dissertation, routes of administration will be given priority as logically; the formulation technology depends critically upon the route of administration for complex formulation in their critical application.

1.1 Routes of Administration

According to the U.S. Food and Drug Agency (FDA, 2016), there are many routes of administration for a pharmaceutical product/formulation in a human body and can be referenced from their guidelines from data (monographs) standard manual, (Food and Drug Agency, USA. Routes of Administration- Guidelines). From these guidelines, a comprehensive table in the resource reference indicates these various routes. To one end, the routes of administration in FDA data bases focus a buccal intake towards interstitial/intravascular administration and from nasal/sublingual until vaginal/urethral administration etc. In the literature, there are various ways

to classify these routes depending upon the application of complex formulations (Food and Drug Agency, USA). However, a characteristic distribution of these administration routes to quantify into their system wise classification would be helpful in describing systemic routes of administration. Here in this dissertation, systemic classification of different routes of administration has been followed as it is indicated in the chart.

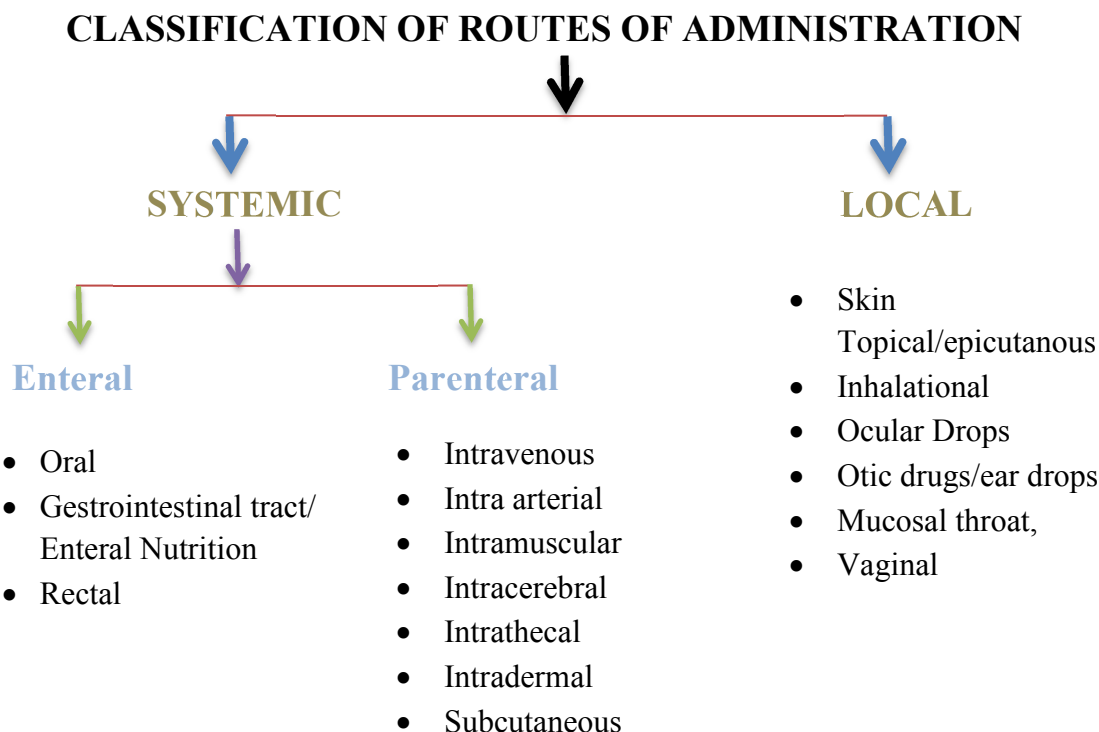


Figure 1.1: Schematic classification of routes of administration for pharmaceutical complex formulations.

According to this chart, the systemic classification would mainly concentrate on enteral and parenteral administration of the formulation routes. By definition, enteral belongs to those administration routes whereby gastrointestinal tract is used for the drug delivery while all other delivery routes concerned parenteral tracts (administration of medicines *NursingTimes.net*. 2007).

A clear line of action must be made here with reference to the research work carried out within the frame work of UHU - Fresenius Kabi collaboration project. It must be taken into account that the main focus of this thesis throughout the research period was strictly on *parenteral formulations* through intravenous administration, commonly known as IV (Intravenous) emulsions. In this way, subsequent discussions ahead in this dissertation will be made with reference to the parenteral administration of the complex formulations for IV administration of formulations.

1.2 Parenteral Formulations

As discussed earlier in section 1.1 about the advent of parenteral formulations, the routes of administration concerning other than enteral are termed parenteral (The term “para – enteron” (beside the intestine) refers basically, other than enteral route (Bryans et al. 2016). The advantages and drawbacks of parenteral route of administration are out of the scope of this research, commonly known as IV (intravenous) emulsions, whereby the focus is maintained mainly on the pros and cons of the formulation technology for the development of parenteral formulations. Following are the several different parenteral formulations product available commercially for parenteral administration. (Linda, 2012; Jose et al. 2016; FDA, 2016)

- Solutions (ready for injection)
- Soluble and insoluble dry products, freeze dried or powder filled (ready to combine with a solvent prior to administration)
- Suspensions (ready for injection)
- Emulsions (usually o/w origin or multi- nature i.e. w/o/w)
- Liquid concentrates (dilution prior to administration)

The presence of excipients, sterility and an absence of endotoxins made them useful for parenteral administration. For this, very strict and stringent regulatory conditions must be abided by all manufacturers from the enforcing authorities to conform regulations.

Solutions are injectable parenteral products in which the active drug along with excipients and co-solvents are completely dissolved in carrier vehicle, water. It is the most abundantly used commercial parenteral administered product. Liquid concentrates are also solutions for injection but are in available, transported and marketed as concentrated liquids those are ready to administer with a dilution solvent. Similarly, a suspension contains an active ingredient in an insoluble state with its carrier vehicle. Depending upon the drug particle size, it can never be administered parenterally through intravenous method as the suspended medium may block the blood capillaries during its route of travel in stream (USP <729>, Driscoll et al. 2006; Driscoll, 2005). Suspensions are complex formulations containing drug crystals that tend to precipitate at the bottom; extreme shaking before their administration is necessary to disperse the active part back in the carrier medium. Worth to comment here, regarding formulations of suspensions that a structured vehicle might be designed during the formulation of carrier liquid so as to restrict the movement of active drug held within the structure of the vehicle, suppressing its precipitation.

Dried mass, in the form of soluble lyophilized powder and insoluble (product) material is another parenteral infusion that is ready to be injected after dissolving in a solvent. Vitamins and essentials, prepared with lyophilization process are dissolved in infusion water prior to their

parenteral administration. There are a lot of advancements related to utilization of lyophilization process in modern era of novel formulations and Fresenius Kabi complex formulation department has designed and formulated state of the art lyophilized novel commercial products those are under production at different business units.

Finally, in parenteral formulations, when a water insoluble active ingredient (drug) is desired for a rapid onset of pharmaceutical action, an emulsion is the best suitable alternative. An emulsion is a thermodynamically unstable two-phase system consisting of at least two immiscible liquids, one of which is dispersed in the form of small droplets throughout the other with an emulsifying agent. The dispersed liquid is known as the internal or discontinuous phase, whereas the dispersion medium is known as the external or continuous phase (unc.edu/pharmlabs). Emulsions could act as a form of encapsulation of active drugs (oily phase) within the drop of a carrier liquid. Continuous phase carrying drug in the form of dissolution, dispersion/suspension and/or its intelligent association with carrier drops is scientifically called encapsulation. Depending upon the size of encapsulated drops, the formulations are called micro- or nano-encapsulation and hence on similar basis a micro or nano emulsion.

Emulsions may be conveniently classified according to the distribution of the oil and aqueous phases. Simply, a system which consists of oil droplets dispersed in an aqueous phase is called an oil-in-water or O/W emulsion (e.g. mayonnaise, milk, cream, soups and sauces). An o/w emulsion is generally formed if the aqueous phase constitutes $> 45\%$ of the total weight, and a hydrophilic emulsifier is used (McClements, 2005; Christoph, 1998). Conversely, where water or aqueous solutions are dispersed in an oleaginous medium, the system is known as water-in-oil (w/o) emulsion (e.g. margarine, butter and spreads). W/O emulsions are generally formed if the aqueous phase constitutes $< 45\%$ of the total weight and a lipophilic emulsifier is used (Christoph, 1998). There are other types too known as multiple emulsions having complex systems containing O/W/O or W/O/W types that can also be prepared (McClements, 1999). This thesis is mainly concerned with the microencapsulation and/or emulsions preparation and the technology for designing novel emulsifier systems in developing parenteral formulations.

Schematically, Figure 1.2 indicates different parenteral routes of administration in a self-explanatory way. Subcutaneous, intramuscular, intravenous (IV), transdermal and implantation are the different routes of parenteral drug application/administration.

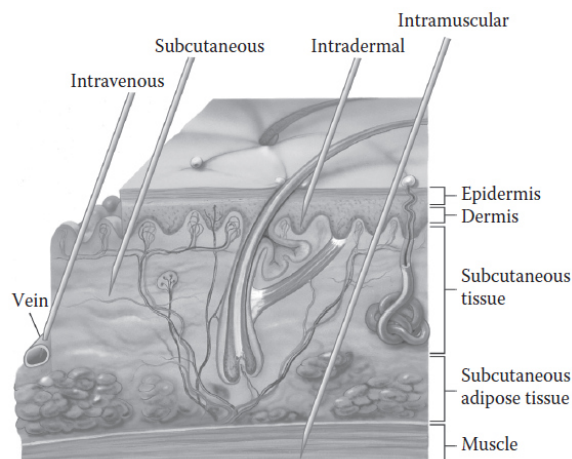


Figure 1.2: Different routes of administration of pharmaceutical drugs (Jose et al. 2016)

1.3 Constituents of oil/water emulsions

Emulsions preparation related to the scope of this thesis is mainly intravenous (IV) type. Emulsions are prepared when two totally immiscible liquids (usually oil and water) are converted into one, with one present as small spherical droplets within the other (McClements, 1999). The magic of this undissolved combination was served by an interfacial layer between the two phases which is occupied by some necessary surfactant material within an emulsion (Friberg et al., 2004; Walstra, 2003). The substance that makes up the droplets in an emulsion is referred to as the dispersed or internal phase; whereas the substance that makes up the surrounding liquid is called the continuous or external phase (McClements, 1999). It is also claimed that the trend of the diameter of these droplets, in most foods, usually lie somewhere between 0.1 and 100 μm (Dickinson, 1992). The concentration of droplets in an emulsion is usually described in terms of the dispersed phase volume fraction (ϕ).

Typical composition of parenteral IV (intravenous) emulsions is as follows. .

- Active drug
- Emulsifiers
- Aqueous phase
- pH controllers- Buffer
- Tonicity adjusters
- Preservatives/stabilizers
- Oily phase
- Antioxidant
- Chelating agents
- Inert gases

There are few necessary constituents of a parenteral nutrition (PN) depending upon nutritional requirements termed generally, in pharmaceutical product formulations, as macro or micro requirements. Macro nutrients of a parenteral product formulation are proteins, carbohydrates and essentially fats. Similarly, micronutrients of a parenteral nutrition, if present in any infusion, constitute basically a specialized product formulation named Total Parenteral Nutrition (TPN). TPN is a nutrition whereby all nutritional requirements are met by only parenteral route and therefore, are out of scope of this research work. However, it will be adequate to name only the essential micronutrients concerning any TPN. Electrolytes, vitamins, minerals and trace elements are the essential constituents of a micro nutrition in TPNs. A very important information in reference to TPNs is that an o/w emulsion is an integrated part of these nutritious products and hence an important constitution worth interesting in relation to the current research work.

Lipid emulsion is a soluble form of fat that allows it to be infused safely in the blood stream (Driscoll et al. 2001 & Mirtallo et al. 2010: 2004). What lipid emulsions provide during a nutritional support is basically essential fatty acids (EFA). Essential fatty acids are the fatty acids required by the human body which can only be synthesized once provided from external source and they cannot be produced by the internal metabolism of the body. Poly unsaturated fatty acids (abbreviated shortly as PUFA) are the most common example of EFA (Linda, 2013). Also, different types of these PUFAs contained in oily phase of a lipid emulsion have an impact on the PN (Shamsuddin, 2005; Kathleen et al, 2005). Omega – 3 (ω -3), Omega – 6 (ω -6) and Omega – 9 (ω -9) are different types of PUFAs present in different proportions, depending upon type and mixtures of oils in any emulsion, having different impact on nutritional characteristics of a PN (Adolph et al. 2009; Fell et al. 2015). Table 1.1 is reproduced from some of the commercial lipid emulsions containing different oils with different nutritional values for ω - ingredients of these oils (Fell et al. 2015). These oils are important nutritional oils used in commercial lipid emulsion products. The same oils are used in next sections of this thesis in production of state-of-the-art production of parenteral emulsions using novel/different emulsifiers.

The types of oils contained in any emulsion (parenteral) affects the dosage of the intravenous (IV) drug in the form of PUFAs and saturated FAs particular loadings. Oil types and the emulsions containing each of these oils expressed in Table 1.1 have significance in the total fat intake during IV injection. Also, these are the oils commercially available for most of the lipid emulsions in pharmaceutical products. In this way, most of the oils used in the current research are the ones described in Table 1.1 unless specified otherwise, in particular.

Table 1.1: Typical composition of FAs of different lipid emulsions (Ferrie, 2015)

Emulsions based on oil types	Fatty Acids (FA): Mono, Poly- unsaturated and saturated				
	ω - 3 PUFA	ω - 6 PUFA	ω - 9 MUFA	Saturated	MCT (medium chain triglycerides)
Soybean oil emulsions	5%	60%	20%	15%	
Olive-soybean oil mixture	3%	20%	60%	17%	
Fish oil emulsions	60%	10%	10%	20%	
Soy/MCT/Olive/Fish multi oil emulsions	10%	20%	30%	10%	30%

Before heading towards the description of constituents of a lipid (intravenous- parenteral) emulsion, worthwhile to note that there are following specifications that must be met by these intravenous (IV) formulations imposed by a competent regulatory authority e.g. FDA.

- Sterility – must be free from microorganisms: yeast, bacteria and viruses, for example.
- Free particles presence, must be limited to a certain oversize particles
- Endotoxin free – free from highly toxic molecule coming from dead cell wall of gram negative bacteria.
- Appearance of the formulation
- pH – as close as possible for a human body natural pH
- Tonicity – must have a salt/ionic balance closer to human body natural tonicity

A detailed and up to date discussion with respect to IV emulsions on the above mentioned specifications is ahead in this section. The main constituents of lipid emulsions are:

1.3.1 Active drug

Active drugs might be of various types but it must be water insoluble because it can be transported to the blood stream in the form of a parenteral solution otherwise. Active drugs for parenteral emulsions were dissolved in oil phase and then droplets were formed with water phase with the help of an emulsifier covering the oil containing drug. The emulsion formation will be discussed in the technological section of emulsion formulations in this section ahead. Active drug of nonpolar nature might be of any type depending upon the target application. For instance, propofol is an active drug used to give local anesthesia to the patients, is dissolved in oily phase to make propofol emulsions.

1.3.2 Emulsifiers

In the formulation of emulsion systems, one normally comes across a very distinguishing agent known as emulsifier (Dickinson, 2003) which gives a kinetic stability to the systems. To form an emulsion that is kinetically stable, one must prevent the instability mechanism, which is described in the next section of this dissertation. This is achieved by having a sufficiently high concentration of emulsifier present during the homogenization process. The emulsifier adsorbs to the surface of the droplets during homogenization forming a protective membrane which prevents them from flocculation or coalescence (McClements, 1999). This agent promotes stabilization by interfacial action (Dickinson, 2003). Many food based emulsifiers are available commercially like egg yolk lecithin, soy lecithin, mustard and sodium phosphates, for example. Many phospholipids are utilized as food grade emulsifiers for their amphoteric character, for example lecithin of different origin etc. Other types of emulsifiers are ionic, cationic and also of nonionic nature (David, et. al., 2006; Linda, 2013)

A variety of fatty acids (e.g., stearic acid), fatty alcohols (e.g., stearyl or cetyl alcohol), and fatty esters (e.g., glyceryl monostearate) serve to stabilize emulsions through their ability to thicken the emulsion. Because these agents have only weak emulsifying properties, they are always use in combination with other emulsifiers and are indicated in section 1.3.6 ahead.

Literature suggests that a system was developed to assist in making systemic decisions about the amounts and types of surfactants needed in stable products. The system is called the HLB (hydrophile-lipophile balance) system and has an arbitrary scale ranging from 1 – 40, whereby useful for the interest of emulsion formulations to be as 1 to 20, for instance. HLB numbers are experimentally determined for the different emulsifiers (William, 1954). If an emulsifier has a low HLB number, there are a low number of hydrophilic groups on the molecule and it will have more of a lipophilic character. For example, the Spans® have low HLB numbers and are oil soluble. Because of their oil soluble character, Spans® will cause the oil phase to predominate and form a w/o emulsion (HLB from 1 to 7 is considered to be w/o emulsifiers, David et. al. 2006). Similarly, the higher HLB number would indicate that the emulsifier has a large number of hydrophilic groups on the molecule and therefore should be more hydrophilic in character. Tweens® have higher HLB numbers and they are water soluble. Because of their water soluble character, Tweens® will cause the water phase to predominate and form an o/w emulsion. (HLB from 8 to 18 is o/w emulsifiers, David et. al. 2006),

It is also suggested to use combinations of emulsifiers that may produce more stable emulsions than using a single emulsifier with the same HLB number. For example, sodium oleate with HLB as high as 18 was intendedly used as a co-emulsifier in this research work. However, different authors have different opinion regarding the role of sodium oleate in formulations. Table 1.2 gives HLB values for various commercially used emulsifiers with their chemical

names readily available for industrial use. The values were reproduced from the herbarie resource center, as appeared in the bibliography with last accessed date.

Table 1.2: HLB values for some commercial emulsifiers.

Emulsifiers Commercial names	Chemical Name	HLB values
Oleic acid	Oleic acid	1.0
Span® 80	Sorbitan monooleate	4.3
Span® 40	Sorbitan monopalmitate	6.7
Soy lecithin	lecithin	9.7 ± 1
Tween® 85	Polyoxyethylene sorbitan trioleate	11
Tween® 40	Polyoxyethylene sorbitan monopalmitate	15.6
Sodium oleate	Sodium oleate	18
Potassium oleate	Potassium oleate	20

In mechanical processing of o/w emulsions, discussed ahead in section 2 of this part, the role of an emulsifier is vital in reduction of particle size from initial stage of pre-emulsion formation with coarse aggregates to homogenization in final stage of emulsion formation with very fine particle size distribution. Exploring new types and origin of emulsifiers and optimizing their amount for an emulsion i.e. parenteral emulsion, is the target of this thesis dissertation whereby utilizing different kinds of marine based emulsifiers having nutritional values too. The amount of these emulsifiers in a particular emulsion is another task dealt within this thesis, for instance.

1.3.3 Aqueous phase

Aqueous phase is the continuous phase in any o/w formulation system. The purpose of an aqueous phase is to transport and protect the oil phase and/or drug carried in it and to reduce down the viscosity of the drug to have an easy intake, especially if it's an IV formulation. Depending upon the type of emulsifier used and with reference to its HLB ratio, the emulsifier can go to either oily phase or an aqueous phase. In general, with respect to HLB, the emulsifier during an o/w emulsion goes to aqueous phase (William, 1949). However, there always might be exceptions, depending upon application and the target formulation. For instance, lecithin with HLB of 8 to 10 (Table 1.2), depending upon its origin, might be dissolved in oil during an o/w emulsion whereas it is easily dissolvable in water too as per its HLB ratio. In this research work concerning different food based emulsifiers and in particular, lecithin, it went to both oil and water phases for its dissolution depending upon target pH of the formulation. Part II deals in

detail with lecithin based emulsifiers and the emulsions formulated using lecithin with different origin.

The particle size of an o/w emulsion depend not only the type and quantity of emulsifier, it also depends upon the percentage of water present as a continuous phase. The less the water phase is, the more the oil/drug loadings are and larger is the particle size with low stability. Conversely, higher water content constitutes a low drug intake, or oil loadings in other sense, while a small particle size results with more stability (Roselena, et. al., 2014). The rule of thumb being, the smaller the particle size is, the higher the stability would be, due to lower particle interaction and inter-particle repulsion with respect to surface charge as a result of optimum emulsifier concentration discussed above (Driscoll et al. 2005; Driscoll 2006). Worth to comment here that in case of clinical o/w emulsions, as was the case within the scope of this dissertation, water for injection (WFI) is always preferred as water phase. WFI has clinical priority and contains controlled minerals with a neutral pH by all means. However, equivalent to WFI, in this research work, freshly extracted milli Q was used for the preparation of these emulsions, as a replacement to commercially available WFI bottles.

1.3.4 pH controllers/Buffering agents

Amongst all constituents of a formulation, the pH of a system is the most important factor concerning any o/w emulsion. In microencapsulation, with intravenous applications, pH is even more important in formulating an emulsion as the requirement of pH for the formulation must fulfill the requirements of the natural pH of the body. pH also has an influence on the controlled delivery of a formulation depending upon the target application. For instance, pH of an oral formulation is totally distinct than a pH of a parenteral formulation. Similarly, for an adult, this range would be different as compared to a similar application for a child or neonatal due to their physiological pH of the body for administration.

For an IV emulsion, Infusion Nursing Society (INS) has suggested guidelines with reference to their pH range for the said application. It is between 5 to 9 pH values (INS manual, 2009). Similarly, the admissible range of IV formulations as per USP method <791> is from 6 to 9 units (USP). Specifically elaborating, a neonatal has an average pH in any PN (parenteral nutrition) with the range from 5.03 to 5.73. The more the pH is, the more the solubility of calcium and phosphate ions is and higher TPN (total parenteral nutrition) effects would be.

Another concern regarding pH controlled range for o/w IV formulations that they are prepared in neutral to 8.5 pH range is due fact to reduce down the rate of undesired reactions because of the interaction of water phase to constituents of emulsifier and/or oily phase (Ferrie et al. 2015). An example of these undesired reactions is hydrolysis reaction, for instance, or formation of free fatty acids (FFA) taking place due to the presence of large amount of water interacting with the oil/emulsifier constituents which itself, logically, cannot be suppressed due to sufficiently high water contents of the o/w emulsions as a continuous/career phase.

A buffer is a salt that is used to control and balance the pH of a particular system in question. However, due to the influence of salt ions to control the pH of water phase, as a buffer solution, after their addition in o/w emulsions, they have an impact on the final zeta potential values of the system; influencing the ζ -potential values effecting stability. So, here in this research work, no buffering salt was used to adjust the pH of the emulsions. However, 1N NaOH solution was used to adjust the pH from 8.3 to 8.7, in general. Also, it should be noted that a very small quantity of sodium oleate was also used during the preparation of these o/w emulsions, in this research work. Due to the presence of oleate ions, sodium oleate regulated the pH of the system to the required values stated above. The role of sodium oleate, however, is not very clear from the literature as Roselena, et. al. (2014) has suggested that sodium oleate functioned as stabilizer and/or solubilizing agent too. Comments regarding sodium oleate concerning these systems are mentioned in section 1.3.6 ahead.

1.3.5 Tonicity adjusters

An isotonic solution/formulation is a formulation that exhibits the same effective osmotic pressure as blood serum does or in other words the solutions that have freezing points at -0.52°C (David, et. al., 2006). Serum osmolality is often loosely stated to be about 300 mOsm/L (Linda A.F: 2013), with a variety of normal bands reported across literature i.e. 275 to 295 mOsm/kg for example (Deepak, et. al., 2008). Delivering parenteral nutrition as an IV formulation, an emulsion must have an osmolality in the range of 300 to 600 mOsm/kg (it should be always less than 900 mOsm/kg, in principle) as per dietitians' association Australia (DAA) manual (Ferrie et al. 2015). Without going into the hypertonic and hypotonic solutions nomenclature, those basically are under and over isotonic characteristics of a solution, by definition, various saline solutions are used for the control of osmolality (in terms isotonicity) of an IV emulsion. For example, NaCl, mannitol and glucose are used as tonicity adjusters.

In this research work, according to pharmacopoeia standards and/or FDA requirements, food based tonicity adjusters were used for the control of osmolality of IV formulations. For this, glycerol with 2.25 and 2.5% was used to adjust the osmolality of formulations in question. Further, Osmolality was measured as a characteristic property of the final emulsions explained in detail in characterization section ahead in this thesis.

1.3.6 Preservatives/Stabilizers

For a prolonged shelf life, preservatives and stabilizers are added in formulations. Preservatives are bacteriostatic to inhibit any microbes accidentally introduced while doses are being withdrawn etc. they are also adjuncts in intermittent heat sterilizations. Sodium azide is used as a preservative in o/w emulsions against microbial growth, for example, when sterility of formulations is not possible due to temperature affects or heat sensitive drugs. Similarly,

depending upon applications and constituents of a formulation, esters of para-hydroxybenzoic acid, methyl and propyl parabens in very small quantities might be used along with phenolic compounds too (Linda, 2013). Esters have however, low aqueous solubility, especially at high pH values.

When stabilizers are used alongside preservatives, their presence might be reduced due to macromolecular structure of polymers added. Also, their presence is restricted with large quantities of surfactants available for micellar inclusions, depending upon the formulations and percentage of oil phase present. For example, emulsifier in a large quantity is available for the formulations containing 10% oil loadings as compared to a 20% formulation due to the fact that same percent of emulsifier is used to emulsify two different oil loadings, a mere formation of micelle in the former emulsions (Mirtallo et al. 2010).

Sodium oleate with very small quantities was used in the systems prepared in this research work. According to Roselena et al. (2014), sodium oleate is commonly used for stabilizing formulations of injectable lipid emulsions, acting as an anionic surfactant and solubilizing agent. Fell et al. (2015) have the same view for the application of sodium oleate in pharmaceutical formulations. Polyethylene glycol was used as a stabilizer in the formation of o/w emulsions for this research work alongside using sodium oleate in addition.

1.3.7 Lipid/oil phase

Oil or lipid phase is the back bone of an o/w emulsion. Oil phase contains the active drug (either itself or added additionally). Lipid phase constitutes a 10 to 30% of oil loadings for an o/w emulsion, in general, depending upon the type and quantity of emulsifier and application of that particular emulsion. The major concern of an aqueous insoluble drug using aqueous media as a carrier was the basis of o/w emulsion so; different oils have been used for the formulation of o/w emulsions containing different drugs/additives. Soy oil, olive and soy oil mixture, fish oil, enriched fish oil and a mixture of soy, MCT, olive and fish oils are typically commercial oils used to formulate o/w emulsions as already indicated in Table 1.1 in IV emulsion section where fatty acid (FAs) contents of emulsions was under discussion.

Active drugs like propofol, is added to the oil phase to formulate state-of-the-art lipid emulsions for a local anesthesia application as already mentioned earlier in active drugs section, 1.3.1. Also, drugs dissolved in oily phase have more bioavailability as it is not in direct contact with water and rather encapsulated in a water droplet. (David, et. al., 2006)

1.3.8 Antioxidants, Chelating agents and inert gas

The possibility of an oxidative decomposition of certain excipients, the oil phase, and some pharmaceutical drugs is possible in o/w emulsions, due to the fact that not only a sufficient quantity of air is incorporated in the liquid during the preparation of the o/w emulsions (ultra turrex), but also because of the large interfacial area present between the oil and water phase

cause the phenomenon. Oxidation is also influenced by the type of oil used in an o/w emulsion. For example, in comparison to fish oil and soy oil, the decomposition is taking place with higher oxidative kinetics with fish oil than in a soy emulsion or even this decomposition would be many folds more when concentrated fish oil will be used. The selection of the appropriate antioxidants depends on such factors as stability, compatibility with the ingredients of the emulsion, toxicity, effectiveness in emulsions, odor, taste, and distribution between the two phases. (Linda, 2013)

Vitamin E, in the form of mixed and α - tocopherols, is used in very small amounts as antioxidants in o/w emulsions and is dissolved in oil phase. For the preparation of o/w formulation systems concerning this research work, vitamin E was used primarily as an antioxidant to inhibit the oxidative decomposition of the constituent oil and formation of free fatty acids (FFA) etc.

The presence of free radicals once formed through decomposition or by hydrolysis reaction might be catalyzed by metals, heat, light and hydrogen ions, for example. A chelating agent is an active ingredient in any solution (o/w emulsion) to sequester heavy metals (ions) in preventing catalysis of oxidation reaction by preferential absorption and/or higher interaction with metal ions radicals. Citric acid and tartaric acids are chelating agents, however, in this research work; no chelating agent was used as such for the formulation of o/w emulsions.

Due to oxidative activity of the o/w emulsions, care must be taken to avoid the interaction of oxygen to the raw ingredients and /or final emulsions too as much as possible. All containers containing either raw materials or the final products must be stored with appropriate purging of inert gas (either with CO₂ or N₂). Nitrogen replacing air will give a lower interaction with oxygen and enhance the stability and/or suppress oxidation.

Further, the product bottles are also purged with nitrogen prior filling the final product, topping off the container with gas after sealing will be an extra step towards conformity of non-oxidation interaction. It is also preferred to use glass sealed ampoules in order to avoid oxygen adsorption prior to the walls of container. Hence, nitrogen was used to purge before sealing the raw ingredient's bottles and also the bottles of produced emulsions within this research work

1.4. Stability of o/w emulsions

All of the above parameters and factors discussed in section 1.3 were the base of a stable emulsion, provided the fact that they must be followed or abide by concerning the stability of an o/w parenteral emulsion. In this section, stability of emulsions will be discussed prior to the processes discussions on how emulsions are formed? It is clear from the explanation above that not considering any of the factors mentioned in section 1.3, instability will result. However, this result will be time dependent and will emerge according to the severity of protocol not followed.

In the following section, various mechanisms of instability for an o/w emulsion are discussed as indicated in literature.

1.4.1. Mechanisms of instability

The term “emulsion in/stability” is broadly used to describe the ability of an emulsion to resist changes in its properties with time. A number of the most important physical mechanisms responsible for the instability of emulsions are shown schematically in Fig. 1.3. Emulsions are thermodynamically unstable systems and they tend to break down over time due to not only an unfavorable contact between oil and water molecules but alongside there is a consequence of their physical structures in having a variety of physico-chemical mechanism (Friberg, 1986). These mechanisms involve gravitational separation, creaming, flocculation and Ostwald’s ripening as explained by McClements (1999; 2005) eventually leading to complete phase separation (McClements, 2005). It is possible to form emulsions that are kinetically stable (metastable) for a reasonable period of time while kinetic stability over the lifetime of the product is usually achieved by the addition of proteins or small-molecule surfactants. The presence of adsorbed surfactant molecules lowers the interfacial tension between the oil and water phases. When the interfacial energy is minimized making the interfacial area between oil and water as small as possible, the two phases could possibly immerse in one another forming an emulsion (Friberg et al., 2004).

Many surfactants (e.g. phospholipids) do not simply reduce interfacial tension but actively inhibit coalescence by altering the viscoelastic properties of the interface. The adsorbed material can also prevent the close approach of oil droplets by causing the surfaces to have sufficient charge to repel one another or by creating an extended surface layer, which also prohibits close approach.

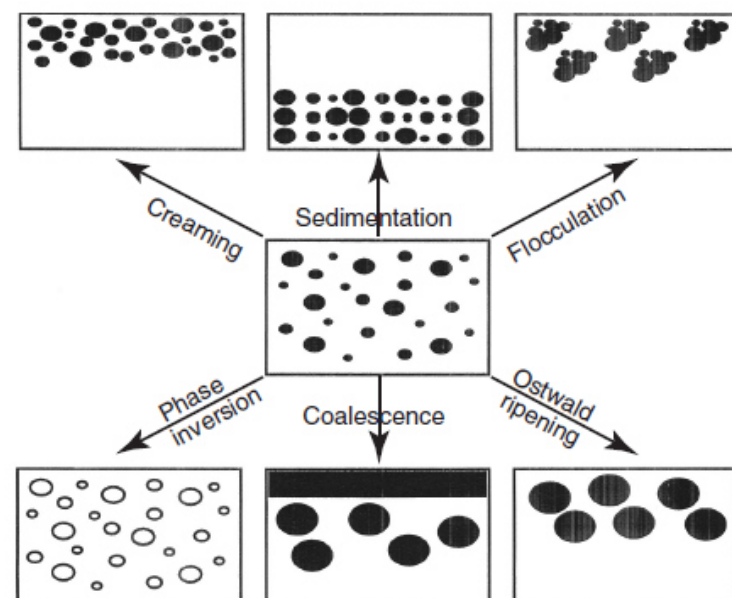


Figure 1.3: Schematic of most common emulsion’s instability mechanisms (Tharwat, 2013)

There are physical mechanisms of emulsion instability which can be found in the literature as was understood and described by McClements (1999). Creaming and sedimentation are both forms of gravitational separation. Creaming describes the upward movement of droplets due to the fact that they have a lower density than the surrounding liquid, whereas sedimentation describes the downward movement of droplets due to the fact that they have a higher density than the surrounding liquid. Flocculation and coalescence are both types of droplet aggregation. Flocculation occurs when two or more droplets come together to form an aggregate in which the droplets retain their individual integrity, whereas coalescence is the process where two or more droplets merge together to form a single larger droplet. Extensive droplet coalescence can eventually lead to the formation of a separate layer of oil on top of a sample, which is known as “oiling-off”. Phase inversion is the process whereby an oil-in-water emulsion is converted into a water-in-oil emulsion or vice versa.

The systems concerned in this thesis are oil/water emulsions comprising major constituent as water (continuous phase), around 80% (w/w) basically. To investigate the role of aqueous phase composition on the stability criteria of oil-in-water emulsions, a great deal of investigations has been carried out by many researchers (Hunter, 2001). The aqueous phase in its major role may affect o/w formulations in defining pH of system, the amount of mineral ions present, surface active molecules dissolved in it. In an emulsion, when the dispersed phase (oil) droplets encounter each other, they are attracted by van der Waals forces. In order to reduce the coalescence between droplets of an unstable system like emulsions, the force between them must be modified and this can be accomplished by adding an appropriate stabilizer which is adsorbed at the droplet surfaces that introduce a repulsive force to provide an energy barrier and mechanical barrier at the interface to prevent coalescence of the drops Kong et al. (2001).

To accomplish this barrier, proteins such as egg protein and soy protein isolate, and polar lipids such as lecithin are some of the more commonly used stabilizers in food emulsions. While they were drastically used in stabilizing emulsions, their function in enhancing stability is not well understood as discussed by various authors (Dickinson 2003).

2. Products Formulation Technology

Products formulation technology is the state-of-the-art technology developed specifically for the indigenous production of up to date products for specialty application. According to methods explained in literature, it may involve a single step in the formation of emulsions or a number of consecutive steps depending upon the target goal (McClements, 1999). The production and control of submicron emulsions with optimization of the process parameters, with a narrow size distribution of oil droplets, is the key to industrial emulsions (food and pharmaceutical applications). In this way, as initially described, the process of converting two immiscible liquids into an emulsion is known as homogenization. To produce these emulsions either a large amount of energy or surfactant or a combination of both is required. The low-energy homogenizer (e.g. a high speed blender and/or rotor stator system, usually called Ultra-Turrax) is used during the primary step to intermingle finitely the separated oil and water phases into a coarse emulsion. The high-energy homogenizer (e.g. high pressure valve system- very wide variety of systems is available) is then used to reduce droplet size and promote an effective emulsification process

The detailed technology of product formulation is discussed below in this section with the pros and cons of mechanical energy and chemical interaction requirements of the processes. An acknowledgement had already been made to Fresenius Kabi for its direction in production and control of o/w emulsion preparations and also for material procurement.

2.1 O/W emulsions preparation methods

This section deals with the preparation methods of the formulations, which were discussed in detail in constituents of formulation section (section 1.3).

Preparation techniques used for emulsion preparations were not only as per description in the literature (David et. al, 2006; Linda, 2013 etc.) but guidance from Fresenius- Kabi were also considered due to experience involved in good manufacturing practice (GMP).protocols followed by the pharmaceutical giant and acceptance from USP and international pharmacopeia standards etc.

Also, from literature, the idea was replicated from various preparation techniques for food based emulsions as discussed extensively by Tokle and McClements (2011), Nagasaka and Ishii (2001), Krickau et al. (2007), Knoth et al. (2005) and Choi et al. (2011). Specific to the preservative treatment, the protocol followed by Watrobska-Swietlikowska and Sznitowska (2006) was the most relevant to the current work.

2.1.1 Aqueous phase preparation

DI water was heated on heating stage with temperature controller plate and putting an electromagnetic agitator inside DI water container during heating. The temperature was regulated at 60 °C with agitation no more than 300/500 rpm. Different origin of lecithin as emulsifier constituent of emulsions, with its HLB value of 8 ± 1 , was either dissolved in aqueous phase or in most of the cases, in oil phase, within the scope of this research.

Polyethylene glycol, PEG, if necessary, was added at room temperature in aqueous phase. Glycerol was added with respective percentage for each formulation, to control the tonicity of the solution, when the temperature reached at 40-45 °C, as glycerol is hygroscopic in nature, it took no time in dissolution at that temperature.

Sodium oleate, if added in formulations, had the next turn for dissolution at approx. same temperature (45 °C), after glycerol was added. Sodium oleate regulated the pH of the water phase too with values ranging from 8.5 to 9 pH. Care was taken in reducing the agitation of water phase to minimize the foam formation due to oleate ions present in solution. The water phase was kept agitating till the temperature was increased to 60 °C. The aqueous phase was retained at that temperature for about 5/7 mins to homogenize the contents completely and then transferred to Ultra Turrax for pre-emulsion formation step discussed ahead in section 2.1.3.

2.1.2 Oil phase preparation

There were different types of oils used in this research work as indicated in Table 1.1 or indicated in respective materials and methods section of emulsion formations in respective parts of this thesis. Percentage of these oils for each formulation is presented in respective sections of emulsion production in corresponding parts of this research work too. Oil phase consisted of respective oil and/or mixture of oils and vitamin E, for instance. The mixture was heated in a way that minimized prolonged heating by efficiently heating the phase, so as to negate degradation of oils at longer time exposure at higher temperatures. Temperature of the agitator was kept at 60 °C and the oil phase was heated after aqueous phase to reduce the time of heated oil exposure to lab atmosphere (i.e. oxygen exposure). When the mixture reached temperature around 55 °C, it was kept for 3-5 mins at the same temperature and then transferred to Ultra

Turrax for pre-emulsion formation (section 2.1.3). No active drug was added in oil phase within the scope of this research work.

2.1.3 Coarse emulsions: Primary homogenization

Hand held high speed blender of rotor stator type (Ultra-Turrax) was always used to make coarse emulsion in quantities of about 1000 gms (1L equivalent) oil/water samples. Aqueous phase was gently agitated with Ultra Turrax at 55-60 °C, placing rotor at its surface to minimize foam formation from sodium oleate, if added. Then pre-heated oil phase was added in it and velocity was increased slowly from min to max in an interval of 2 mins. After addition of oil phase thoroughly, 10 mins of strong agitation was carried out for all the formulations. To remove foam from the pre-emulsion, the container was kept in a water bath at 50-55 °C for 10-15 mins until the foam was removed. The volume was adjusted to 1L if necessarily observed reduced after foam removal. The coarse emulsions were checked and balanced with required pH value of 8.5 after processing.

2.1.4 Fine emulsions: Secondary homogenization

Two types of High-pressure homogenizers were used in this research work. Micro-fluidizer model 110L (Microfluidics Corporation Newton, MA. USA) and Twin panda from GEA Niro-Soavi was used to homogenize pH controlled pre-emulsions. Pneumatically driven micro-fluidizer homogenizer was used in the second part of this thesis for the emulsions preparation while compressor motor driven, twin panda homogenizer was used in the rest of this research work. The working pressure of 500 bar (7500 psi) was used for both cases and samples were homogenized to a defined number of 5 to 6 cycles to make fine emulsions.

Micro-fluidizer contains an interaction capsule having “Y” shaped capillary with a minimum capillary diameter of 75 µm. The homogenizer was made function at room temperature for the processing. While Twin Panda homogenizer was pre-heated with DI water at 55 °C, before processing. It has interaction chamber with two stages, the pressure of each was adjusted one after the other, i.e. first stage pressure was set at 50-60 bars while 2nd stage was adjusted to make 500 bars, overall. After 4th homogenization cycle, sample was extracted for characterization.

Finally, after all passes (5 or 6, depending upon homogenizer), a part of emulsion was extracted for characterization, i.e. particle size distribution (PSD) and microscopy, while rest was pH controlled and subjected to autoclave treatment after cooling down to room temperature. All the readings were recorded along with observations for each cycle (temperature and pressure) and archived for future reference. Homogenizer was then cleaned with standard protocol to avoid contamination. Photographs, if necessary during processing, were also shot as observations and recorded for further reference.

2.1.5 Autoclave treatment

Sterilization was carried out for all the emulsions after homogenization treatment at a defined temperature of 121 °C with a time cycle of 15 mins [European Pharmacopoeia standard 5.0] by using steam autoclave for sterilization. Samples were withdrawn right after sterilization as soon as pressure reached atmospheric value without giving any tempering effect. In order to demonstrate strict sterilization conditions, samples from each formulation were subjected to autoclave treatment in 8 different infusion glass (sealed) vials with 100ml volume and nitrogen purging before sealing, to make an intensive stability study for 4 consecutive weeks.

Other than characterization sequence for Part II of this thesis, whereby only creaming test was evaluated at different times during stability check, in part III of this dissertation, the samples after autoclave were stored in following fashion respectively.

3 vials each for 25 and 40 °C while 1 vial at 5 °C (Total 7 vials stored at mentioned temperatures).

Each sealed glass vial was properly labeled for identification with a standard method disseminated by Fresenius- Kabi along with date of manufacture and storage temperature. The characterization cycle was 1st and 2nd week stability check at 25 and 40 °C while after 4 weeks; stability check was made at 5, 25 and 40 °C respectively. The remaining vial (No. 8th) was characterized after autoclave treatment at time 0, due to the fact that ζ - potential of the emulsions does not change after this treatment (Schwarz and Mehnert 1999 & Heiati et al., 1998)

2.2. Characterization

Formulation preparation is an easy task to start with but to comply with the USP (United States Pharmacopeia) and/or other pharmacopeia standards is one of the main targets of a formulation technologist. Characterization techniques, in this sense, are the core values in order to conform any formulation with respect to international and standardized compliance while predicting the stability of any formulation.

It has been discussed earlier in section 1.4 regarding the instability mechanisms involved during the course of emulsion life, it is very obvious to quote that focus of every emulsion production is to formulate a stable product giving worth a long and stable shelf life. Creaming, flocculation, Ostwald's ripening, aggregation, phase inversion and phase separation etc. were the instabilities during the long run, an emulsion might encounter. Characterization, on account of certain methods and standardized values, predicts the stability of these formulations.

For emulsions, there are variety of characterization techniques involved to confirm and predict the stability of any formulation, failing to encounter those specialized values predicted by that certain technique would conversely mean an instable formulation or vice versa. For these

stability studies requirements, accelerated and slow stability criteria was also registered and characterized for the emulsions in question within this study. For slow instability phenomenon to occur, the samples were stored at 5 °C and characterized after 4 weeks of time while for accelerated study, samples were stored at 40 °C and were characterized every week (Bryans et al. 2016)

Here, in this project work, the techniques involved in characterization of o/w emulsions were principally taken from USP and other pharmacopeia standards i.e. visual observation for any free oil drops along with microscopic assessment, if necessary, particle size distribution analysis, osmolality, PFAT5 and zeta potential measurements. Density was also checked for the fresh sample and samples after 4 weeks of stability, if required. The comments regarding the qualification criteria were already made in constituents of formulation section 1.1.

Briefly, here in this section, ways to characterize the emulsions are discussed and reference will be made to all these techniques used to characterize the formulations ahead in subsequent parts of this thesis.

2.2.1 Control of pH

pH was controlled during processing and during the length of stability of formulations. The bench mark was to control and get the pH values as close to 8.3 to 8.7 preferable for IV emulsions as per USP <791> (section 1.3.4).

Sodium oleate added DI water was pH controlled after pre-emulsion stage and was automatically adjusted at 8.4-8.6 values due to its buffering effect. pH was only checked and balanced for the systems after pre-emulsion and homogenization stage just to have a tighter control on the prepared formulations. For balancing, 1N NaOH solution was used to adjust the pH of formulations. In case of DI water was used without sodium oleate, preference was given to make a pH balance higher than 9 pH value before pre-emulsion stage. During pre-emulsion stage and after 6th homogenization cycle, pH was again controlled and balanced while after autoclave treatment, pH was only recorded and reported as such.

2.2.2 Creaming test

The prepared fine emulsions and sterilized samples were visually checked for any cream formed during the course of time. The sterilized samples were marked if there is any visual oil content at the top of emulsions after 24 h of treatment. In this study, observations of separation trends as reviewed by Roland et al. (2003) and McClements (2005) would be followed as reference. The appearance of characteristic emulsifier oil layer in emulsions, if any (as would be the case for part III), was given a special protocol and emulsions were not marked unstable if there was an evidence of that layer. In this way, only naked oil drops were reported, rather. Florescent lamp was used for visual observation, if needed, and evidence was collected in the form of

photographs for any future reference during the stability study. PFAT5 evaluation, explained ahead, was basically the technique used to characterize any remarkable free oil contents in the formulations, characteristically.

2.2.3 Particle size distribution (PSD)

Particle size distribution of any oil-in-water emulsion indicates how well oil drops are dispersed within the matrix. Generally, a smaller droplet size and narrow distribution would mean a stable emulsion and hence a long shelf life. United States Pharmacopeia (USP) chapter <729> defines the allowable limit to that PSD characterization which is less than 0.5 μm in any IV emulsion. The size of IV lipid emulsions droplets (200 – 600 nm) should remain well below the diameter of capillaries to avoid vascular occlusion (Wanten, 2015). The particle size of droplet in the emulsions was measured by dynamic light scattering devices nowadays and here in this research work, PSD was also characterized by one of these types of devices described in materials and methods section of part II. It should be noted that only homogenized and autoclave treated samples were characterized for PSD measurement. The emulsions were diluted in distilled water prior to measurements to avoid multiple scattering affects. PSD was always presented and calculated as volume distributed mean $D_{4,3}$ and for plotting the data, OriginLab- Pro was used after extracting the raw data from the respective software. All samples were characterized on the same day of preparation after processing. Each respective PSD characterization of the samples with stability study is explained in respective parts of this thesis for detailed explanation.

2.2.4 Zeta potential

The electric charge (ζ - potential) of the emulsion droplets was measured using a micro-electrophoresis commercial instrument. The ζ - potential was calculated from measurements of the electrophoretic mobility of the droplets in an applied oscillating electric field using laser Doppler velocimetry. Smoluchowski model was always used to convert the mobility into ζ -potential automatically from the integrated software of the equipment in question. One of the drawbacks of using electrophoresis to measure the zeta potential was the dilution of sample, which can cause modification in both emulsion drop size and surface charge. Different authors have dealt this problem differently by following a specific protocol for dilution in all of their measurements (Roland et al., 2003 and Choi et al., 2011).

In order to neglect the effect of concentration/dilution on the zeta potential of a sample, protocol was established in measuring zeta potential as explained by Tantra et al. (2010). This effect is especially important when the sample particle size is in a nano-size range. The samples were diluted in their respective pH controlled solutions (consult respective part of this thesis) to make zeta potential measurements. Repetition was made in order to get maximum concentration of

samples within working range of the instrument. The idea was, to get an appropriate representation of the sample within diluted emulsion and not like PSD measurement where only a turbid emulsion sample may serve the purpose. Emulsion's zeta potential values from $\geq \pm 30$ mV were considered stable as per DLVO theory concerning stability of formulations with respect to interaction of particles within the vicinity of one another in a colloidal solution.

2.2.5 PFAT5 values

PFAT5 is defined by United States Pharmacopeia Chapter <729> as the "percentage of fat residing in globules larger than 5 μm (PFAT5) for a given lipid injectable emulsion [is] not to exceed 0.05%" [USP <729>] (Driscoll et al., 2006; Driscoll, 2005). PFAT5 is a measure of free oil droplets within an emulsion and is a scientific way of characterizing and/or quantifying the presence of oil layer/drops apart from visual observations with the help of fluorescent light. The PFAT5 parameter has emerged as the only validated measure of lipid stability. There are clinical consequences of using lipids that exceed the PFAT5 limit, one of them being vascular occlusion. This parameter is applicable to both manufactured and compounded lipid preparations. The clinician should be aware of the limitations of much of the literature concerning the lipid stability assessment.

Accusizer is the only commercially available equipment with different models for the characterization of PFAT5 values. Accusizer was used in the facilities of Fresenius Kabi in Bad Homburg only; the values presented in this thesis are directly quoted from Accusizer at Fresenius facility. Detailed discussion on functioning and analysis of Accusizer is ahead in part III of this thesis.

2.2.6 Osmolality

Osmolality is a measure of how much one substance is dissolved in another substance. The greater the concentration of the substance dissolved, the higher the osmolality. For emulsions used for parenteral applications, osmolality is a very important factor from scientific and regulatory stand point as the lipids have to be dissolved in blood stream taking intravenous path (Deepak et al. 2007). US Pharmacopeia "chapter <785>" describes the regulatory aspects in conformity of a lipid emulsion as it has been mentioned in qualification of formulations earlier in this section. Osmolality can be measured on an analytical instrument called an Osmometer. The values from 288 to 310 mOsm/kg were considered within range as per USP <785>. The Osmometer was only used in facilities of Fresenius Kabi in Bad- homburg and the values were reported as such, if necessary.

2.2.7 Density

Density was measured in the Sample emulsions at a calibrated temperature of 20 °C Density measurements of freshly prepared autoclave sample was carried out in order to get an idea about the density of the formulation and how it deviated from the actual density of water at 1 gm/cm³.

2.2.8 Microscopy

Optical microscopy was carried out for formulations of interest while increasing or decreasing the emulsifier amount and micrographs were taken after processing and/or after autoclave treatment as well. However, at different temperatures, from 5 to 25 and 40 °C during stability period, only interesting formulations were considered for microscopic analysis. Minimum 2 photographs of each sample was taken from different sides and recorded for referenced use and report in subsequent results and discussion section. With PSD below 0.5 µm, it was obvious that at small magnification, a micro/nano emulsion drops cannot be seen, however, just to get visual idea for all practical purposes, microscopic analysis was carried out to evaluate only free oil drops, if any

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Part II

**Formulation and characterization of o/w emulsions stabilized with plant
and egg origin phospholipids – commercial lecithin**

1. Lecithin: Commercial food based emulsifiers:

In relation to different drug delivery methods as well as various routes of administration available, a pharmaceutical product known as o/w emulsion is of great interest and more importantly, a subject of this research work too. Designing an intravenously administered o/w emulsion, an emulsifier comes into act in order to play its vital role in the organization of this thermodynamically unstable but kinetically stable formulation, i.e. macroemulsions (Schubert, & Armbroster, 1992). Emulsifiers, as the most important entity of o/w emulsions, have already been discussed in part I of this thesis in detail and here in this part, the emphasis will be given on a particular type of emulsifier, lecithin, used commercially for the formulation of o/w emulsions.

It was of the great concern and important milestone for the formulation technologists when lecithin and its derivatives, phospholipids for instance, were recognized as “GRASS “generally recognized as safe substances” (US Food and Drug Administration, 1979) for the food and pharmaceutical applications. By the advent of GRASS, lecithin was used since then in various commercial products for food and nutraceutical applications and in a similar way, in pharmaceutical industry too as phospholipids/emulsifiers.

Lecithin became the choice of stabilizers for injectable emulsions since it was biocompatible and biodegradable too. Lecithin contains naturally heterogeneous mixtures of polar and neutral phospholipids obtained from different animal and vegetable sources (Szuhaj, & Sipos, 1989). It mainly contains amphoteric phospholipids, like; phosphatidylcholine and phosphatidylethanolamine, which are uncharged at physiological pH (7.4). Apart from them, some negative phospholipids may also be present in lecithin, depending upon the origin and source of extraction crop and processing (Roselena, et. al. 2014). This heterogeneity is extremely beneficial for its role as an emulsifier because of the fluidity of the interfacial film as compared to a pure phospholipid (Lawrence, 1996) and that was the reason why, lecithin is still used in the commercial o/w emulsions for parenteral IV drug administration.

1.1. Motivation of using different origin of lecithin emulsifiers

The role of an emulsifier in organization of different fat content of an emulsion is very critical. Egg yolk lecithin is used industrially in parenteral nutrition as an emulsifier to stabilize oil in commercial lipid emulsions. The most widely available parenteral lipid emulsion is based on soybean oil, commercially known as Intralipid® with different brand names (Fresenius Kabi AG and Baxter Inc. etc.).

The motivation of searching for new emulsifier options other than commercially used egg lecithin and lipid contents of parenteral emulsions other than soybean oil has been critical in order to counter the adverse clinical effects related to long term supplementation, due to metabolic limitations and immune reactions in critically ill patients. (Shahidi, 2005 and Adan, et al. 1995). It is important to note that adverse reactions for parenteral lipid emulsions are reported to be related to presence of soybean and egg yolk lecithin (Roselena, et. al. 2014). Various drug-food allergy interaction adverse responses can lead to gastrointestinal upset, for example and can go as beyond as anaphylaxis (Wiesner, et. al. 2008).

10% and 20% lipid emulsions available commercially contain said percentages of lipid content but with the same emulsifier percentage (Mirtallo, et. al. 2010). Further, egg yolk phospholipid contains lecithin in the form of phosphatidylcholine amounting to 60 and 90%.

In this way, the ratio of lipid to phospholipids from lecithin differs for the different emulsions, with 20% emulsion having ratio closest to the ratio found in chylomicrons (naturally occurring lipoprotein particles containing triglyceride, proteins, phospholipids and cholesterol: Hussain, 2000 and Sareen, 2013). The complications (risk) related to lipid abnormalities is considered to be lowest when using 20% lipids with the said emulsifier content (Ferrie et al. 2015). Also, it is reported that free phospholipids are much abundantly available in 10% lipid emulsions as compared to 20% emulsions (Mirtallo, et al. 2010). Due to the fact that these phospholipids interfere with the lipoprotein lipase activity, 10% lipid emulsions are cleared slower than 20% emulsions due to presence of these phospholipids as liposome-resembling particles (Kalfarentzos, et. al., 1998).

For negation of these adverse effects reported above in the commercial use of lecithin and lipids, search continues for alternative raw materials so as to find hypoallergenic substitutions for safer parenteral delivery. This part of thesis deals with comparison of emulsions prepared with egg yolk lecithin from Lipoid® and from Fresenius Kabi indigenously prepared egg lecithin to various lecithin from different origin containing different phosphatidylcholine contents. Similarly, lipid contents other than soybean oil with different percentages in final parenteral formulations have been emulsified with the help of various lecithin origin emulsifiers.

1.2. Consideration of fats other than soybean oil in o/w emulsions

Fatty acids (FA) are categorized as saturated, monounsaturated (MUFA) and poly unsaturated fatty acids (PUFA, with 2 or more double bonds) and similarly with the number of carbon chains present in the FA chain i.e. short: 2-4, medium: 6-12 and long: above 14 carbon chains (Vanek, et al. 2012). Further there are three families of PUFA on the basis of ω carbon, which is the carbon atom in the chain where first double bond occurs while counting from methyl end (Vanek, et. al., 2012). The Omega (ω) families are ω -3, ω -6 and ω -9 in PUFA. PUFAs with ω -6 FAs contain linoleic (LA) acid and metabolites, while ω -3 contains α -linolenic acid (ALA) and metabolites (Adolph, et al. 2009).

Polyunsaturated fatty acids cannot be synthesized de novo by higher organisms (mammals) and thus might be considered essential nutrients in the food intake (Adolph. et. al., 2009 & Vanek, et al. 2012). These fatty acids cannot be synthesized because of the lack of denatures enzymes capable of catalyzing double bond formation at the third and sixth bonds from the terminal carbon in long chain FAs. Therefore, ω -3 and ω -6 FAs are essential fatty acids (EFAs) and must be obtained in diet while ω -9 (containing Oleic acid- OA) is a nonessential FA. Parenteral nutrition provides this external source to fulfil the requirement of supplying sufficient FAs to cater the physiological demand for essential fatty acids and modulate pro- inflammatory processes etc.

If PUFAs not supplied in fatty acid source, essential fatty acid deficiency EFAD may cause and hence must be supplied as a primary source in parenteral nutrition (IV Emulsions) as discussed earlier. Soy oil is still the biggest source of PUFA in commercial products from Fresenius Kabi AG or Baxter Inc. commonly known as Intralipid®. It is suggested that due to the preponderance of linoleic acid in soybean oil fat emulsions, as suggested by Hise & Brown, (2007), it affects immune functions and causes infections in critically ill patients and have potentially amplifying inflammatory responses in particularly stressed patients. After sufficiently long use of soybean oil in lipid emulsions, there were concerns regarding higher content of ω -6 fatty acids in these emulsions (higher ω -6 to ω -3 ratio, i.e. 7:1), which might develop pro-inflammatory mediators, such as sepsis and trauma (Furukawa, et al. 2002) and hence sparked the development and use of next generation lipid emulsions

Further, the continued supply of ω -6 from soy bean oil fatty acid source in the 1st generation IV emulsions (Intralipid®, for example) induced a complication in the long term IV therapy hence introducing intestinal failure associated liver disease (IFALD) as discussed by Cober & Teitelbaum (2010). IFALD is associated to liver dysfunction, and reduced omega-6 long chain fatty acid content is a common reason why alternative lipid emulsions must be used commercially (Cober & Teitelbaum, 2010). The cause and mechanism of this disease is yet

unknown but it is suggested that it includes macrophage activation by excess ω -6 polyunsaturated fatty acids (PUFA) from soybean oil lipid emulsions, leading to pro-inflammatory cytokines etc. (Goulet, et al. 2009 & Wanten, 2015). Convincingly, a higher omega-3 to omega-6 ratio is preferred in modern parenteral emulsions while the fat content along with the emulsifiers are managed accordingly (DAA manual, 2011).

In addressing the same, a second generation IV fat emulsions containing long chain triglycerides (soy oil) in a 50:50 mixture of medium chain triglycerides (MCT, derived from esterification of coconut oil, Roselena, et al. (2014) or palm kernel oil etc) were introduced to address the IFALD, in 1984. Similarly, during 1990s, third generation IVFE were introduced and marketed under Baxter Inc. containing mixtures of olive oil with soybean oil in 80:20 ratio.

Various authors including Vanek, et al. (2012) suggested that clinically ω -3 FAs are less pro inflammatory than ω -6 FAs, relatively, and they were of the view that ω -3 FAs have anti-inflammatory effects, rather (Lee, et. al., 2006). Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) are the precursors of ALA in FAs contained in ω -3 acids and metabolites (Lee. et. al., 2009, Adolph. et. al., 2009) those are considered to have anti-inflammatory characteristics with prostanoids and leukotrienes etc. (Lee, et al. 2009). Hise & Brown, (2007) were of the view that these anti-inflammatory inhibitors were obtained from eicosanoids and the multiple double bonds of these poly unsaturated fatty acids (PUFA) from IV fat emulsions serve as precursors to eicosanoids.

In this way, the fourth generation intravenous fat emulsions (IVFE) included fish oil in the fat source either combined with other oils or alone (Vanek, et al. 2012). It must be note that fish oil is specifically rich in ω -3 FAs and is a heavy source of EPA and DHA (Kim, etal. 1983).

Concluding the motivation for the new source of oils in parenteral fat emulsions administered intravenously, a mixture of different oils containing sources of ω -3 and ω -6 was manufactured and marketed by Fresenius under the brand name of SMOF emulsions containing soy, MCT, olive and fish oils in a 3:3:2.5:1.5 ratio. SMOF would be considered in the subsequent section of this thesis. Other sources of lipids are purified fish oil rich in EPA and DHA with a trade name Omegaven® by Fresenius just like Intralipid®, containing only soybean oil, is in the market for quite a few time from now.

The following research work was conducted by preparing the emulsions in different oils and their combinations along with the emulsifiers of various origins.

1.3. Lecithin o/w emulsions:

This part of thesis is dedicated to prepare and characterize lecithin based oil-in-water emulsions. An emphasis was made to make a wider over view on the influence of the type of lecithin used to prepare each type of pH controlled o/w emulsions in different fat sources, i.e. soy and fish oil, for instance, with an objective of considering a simple three component system containing water, oil and an emulsifier (i.e. lecithin of different types and origin). Emulsions were prepared in presence and absence of sodium oleate to establish the correlation for their stability with specific lecithin type in a specific pH environment.

It is obvious that pH is considered to be one of the very important factors of emulsions formation especially related to routes of administration for a particular application, therefore, the pH controlled formulations prepared in the current research would establish the basis of experimental knowledge for subsequent stability studies. IV parenteral formulations were prepared in a series of different pH controlled environment ranges from as low as 2 units with a transition towards neutral pH emulsions (7) and their translation to a high pH value of 9 units. The knowledge developed in the comparison of different lecithin origin o/w emulsions, containing various amounts of phospholipids, at different pH values and the stability regarding these formulations will hence become the basis for the design of new emulsion systems using novel emulsifiers in subsequent sections of this thesis.

2. Materials and Methods

In this section, details regarding the type and quantities of materials used for the formulation of IV emulsions using different origin lecithin and respective oil types are discussed. The ratio of the materials used in oil and water phases during the preparation of respective emulsions is also commented with the scope of methodology adopted for the said preparations. However, methodology regarding the preparation of o/w emulsions has already been discussed in detail in part I.

2.1 Type and amount of lecithin used in emulsion preparation

Different commercial Lipoid ® and Cargill ® lecithin products of various origins were used to prepare o/w emulsions. Lipoid S 75, Lipoid S 45, Lipoid H 50 and Lipoid LPC 20 were made available from Lipoid GmbH (Lipoid AG, Switzerland). Epikuron 200 was attained from Cargill (Cargill Incorporated, Minneapolis, USA). Another Egg based lecithin was kindly sent by Fresenius-Kabi AG with the name PL90. All of the above lecithin samples were used as sent by the supplier without further purification. For convenience, names of each lecithin type and its origin is listed and classified in Table 2.1. Stability study was conducted for emulsions prepared with different types of lecithin at 2, 3, 4, 5, 7, 8 & 9 pH values for given oil loading. The concentration of lecithin was kept at 1.2% (w/w) for all emulsion formulations. The idea for the chosen percentage of lecithin i.e. 1.2% was taken from the work carried upon by Ishii et al. (1990) who reported that minimum 1.2% lecithin provided stability of emulsions in view of PSD and ζ - potential. Similarly Diprivan® contains 1.2% lecithin (Han and Washington, 2005) and the most famous intravenous (IV) commercial emulsion Intralipid®, contains 1.2% lecithin, produced indifferently by Fresenius Kabi AG and Baxter Inc. (Kawaguchi et al. 2008; Roland et al. 2003 and Washington, 1990).

Tables 2.2, 2.3 and 2.4 below, show some composition data of the different lecithin used in this work. This data has been taken from the technical data sheet of each product sent by the supplier for instance. Below presented tables comprise only constituents of lecithin that were of scientific interest and further discussion would be held on emulsion formation and the role of constituents/ingredients of emulsifier during stability on the basis of these constituents etc.

Table 2.1: Types of Lecithin used for the preparation of emulsions. Samples have been marked with the said names in subsequent sections.

Types of Lecithin used for Emulsion preparation	
Soy origin	Lipoid S 75
	Lipoid S 45
	Epikuron 200
Egg origin	Lipoid E
	Egg Lecithin from Fresenius-Kabi AG (PL 90)
Sunflower origin	Lipoid H 50
Rapeseed origin	Lipoid R LPC 20

Table 2.2 : Chemical composition of soy derived lecithin as indicated by the supplier (Lipoid® and Cargill ®)

Fat-Free Soybean Phospholipids				
Lipoid	Phosphatidyl choline	Phosphatidyl ethanolamine	Lysophosphatidyl choline	PUFA (in total fatty acids)
S 75	70%	7-10%	3% max	4-6% α LA
S 45	45% min	10-18%	4% max	4-6% α LA
Epikuron	Phosphatidyl choline	Phosphatidyl ethanolamine	Lysophosphatidyl choline	Fatty acid (in total fatty acids)
200	92%	0-2%	3% max	9% α LA

Table 2.3: Chemical composition of egg derived lecithin as indicated by the supplier.

Egg Phospholipids				
Lipoid®	Phosphatidyl choline	Phosphatidyl ethanolamine	Lysophosphatidyl choline	PUFA (in total fatty acids)
E	70%	12-18%	3% max	1% α LA
Fresenius-Kabi	Phosphatidyl choline	Phosphatidyl ethanolamine	Lysophosphatidyl choline	Free Fatty Acids
PL 90	69.8%	14.6%	1.5%	0.2%

Table 2.4: Chemical composition of Sunflower and Rapeseed derived lecithin as indicated by the supplier (Lipoid®)

Fat free Sunflower Lecithin				
Lipoid	Phosphatidyl choline	Phosphatidyl ethanolamine	Lysophosphatidyl choline	PUFA (in total fatty acids)
H 50	50% min	5% min	-	0.5% α LA
Partially Hydrolyzed Rapeseed Lecithin				
R LPC 20	20% min	-	20% min	2-5% α LA

Sodium oleate was also used as a co-emulsifier/stabilizer in the formulations in a very low amount equal to 0.2% (w/w). Same emulsions were prepared with and without sodium oleate. Unlike lecithin, sodium oleate was not able to get dissolved in oil phase, so it was poured in water at 40-50 °C. Glycerin was not able to aid its dissolution in water and to enhance this, few drops of 1N HCl were added which worked effectively.

2.2 Types of oil and concentration used in emulsion preparation

Different types of oils were used to prepare the emulsion systems i.e. soy oil and fish oil (concentrate and natural- only purified) with a fixed amount of 20% (w/w) concentration in the final emulsions. Commercial soy oil was kindly supplied by Fresenius Kabi Deutschland GmbH and was used directly in this work while fish oil was supplied by Solutex® (Solutex S.A, Madrid, Spain) marked as 6507 and 0765 in the form of concentrate with more than 70% DHA+ EPA (Docosahexaenoic acid and Eicosapentaenoic acid). Allergen free, natural but purified fish oil was supplied by SuanFarma (SuanFarma, Madrid, Spain) containing 35 % DHA + EPA.

2.3 Methodology: Emulsion Preparation

Methodology of emulsions formulation has been described in the materials and methods part of this thesis earlier in first part. With reference to the methodology adopted for the preparation of these emulsions, only brief discussion will be made on the formulations preparation here in this section. For detailed overview, part I of this thesis may be consulted.

2.3.1 Control of pH

pH of the systems was controlled using of 0.1/1 N HCl and 0.1/0.5 N NaOH solutions. Lab pH meter GLP- 21 with electrode 50 14 T (Crison S. A. Barcelona, Spain) was used to check the pH of formulations. Freshly extracted distilled water was used for all the formulations; the pH of distilled water was measured between 6.8 and 6.9 pH values. Only for comparison of results with the buffer solution, however, an acetate buffer was used for acidic pH values (i.e. 2, 3, 4 and 5) while phosphate buffer was used for basic pH (8 and 9). pH of all the solutions were freshly controlled and were immediately used as such. pH was again controlled after pre emulsion stage and during 3rd and 5th homogenization cycle.

2. 3.2 Lecithin in Solution

Protocol developed for lecithin dissolution was constrained to oil only; an exception to this was R LPC 20 lecithin type, as this lecithin was not characteristically able to be dissolved in any oil type (i.e. soy, fish etc.). R LPC 20 was, in this way, dissolved in water for formulation preparations. Freshly distilled water was pH controlled shortly before making formulation. Agimatic E (J. P. Selecta S.A. Spain) was used as an electromagnetic agitator. The agitation temperature was kept in the range between 55-60 °C for fish oil dissolution in order to suppress oxidation at high temperature (fish oil is highly prone to oxidation especially at high temperatures). For dissolution in soy oil, the temperature was set to 70-90 °C depending upon lecithin type. The agitation time and rate depended upon the type of lecithin and also its affinity to dissolve in particular oil type. It normally took 0.5-1 hour to dissolve lecithin in fish oil as compared to soy oil where egg lecithin from Fresenius-Kabi took 5-6 hours at low temperatures. Similarly H-50 sample took longer time to dissolve in soy oil unless its temperature was raised to 90 °C. Sodium oleate is insoluble in oil and is partially soluble in water so it was dissolved in water at 40-50 °C while few drops of 1 N HCl were added for its efficient dissolution. Sodium oleate raised the pH of solution when it was dissolved so the pH of sodium oleate turbid solution was controlled continuously during processing and after.

2.4. Preparation Techniques

Preparation techniques for emulsions were adopted from typical Fresenius protocol for the manufacture and formulation of emulsions, optimized for the said commercial use. However these preparation techniques were scientifically described and discussed extensively by Tokle and McClements (2011), Nagasaka and Ishii (2001), Krickau et al. (2007), Knoth et al. (2005) and Choi et al. (2011). In the current work, the method close to Choi et al. (2011) has been adopted while specific to the preservative treatment, the protocol followed by Watrobska-Swietlikowska and Sznitowska (2006) has been taken.

Preparation techniques have already been described completely in part I of this thesis in methodology section, however, a brief step wise preparation methods for emulsions are discussed here in this section.

2.4.1 Coarse emulsions: Primary homogenization

Hand held high speed blender of rotor stator type- Ultra-Turrax T 50 basic (Ika- Werke GmbH, Staufen, Germany) was used at 10,000 rpm to make 100 gms of coarse emulsion samples for 2m30s for all formulations. More specifically, 1 min oil addition in freshly pH controlled distilled water and the rest time for thorough mixing. The coarse emulsions were checked and balanced with required pH value at room temperature. Special care was taken for sodium oleate co-added emulsions due to the fact that sodium oleate increased the overall pH of solutions. If more acid was added to lower the solution pH, experimental observations indicated that sodium oleate precipitated out of solution if the pH was lowered than from a threshold neutral value.

2.4.2 Fine emulsions: Final homogenization:

High-pressure homogenizer; commercially called Micro-fluidizer model 110L (Microfluidics Corporation Newton, MA. USA) was used to homogenize pH controlled pre-emulsions. Compressed air was used to control the working pressure at 500 bar (7500 psi) and samples were homogenized to a defined number of 5 cycles to make fine emulsions. While at 3rd homogenization cycle, pH was controlled for a strict protocol of pH in emulsions. After 5 passes, the final emulsion was characterized for particle size distribution (PSD) and a part of it was stored at ambient conditions to check creaming/separation etc. However, it is eminent that the microbial growth had taken place during time “t” without a sterilization treatment. The remaining emulsion was subjected to autoclave treatment.

2.4.3 Autoclave treatment:

Sterilization was carried out for all the emulsions after homogenization treatment at a defined temperature of 121 °C with a time cycle of 15 mins as per guidelines from European Pharmacopoeia standard 5.0. Steam autoclave AES-12 (Raypa S. A. Barcelona, Spain) was used to conduct sterilization at maximum pressure of 2 bars. Samples were withdrawn right after sterilization as soon as pressure reached atmospheric value without giving any tempering effect. Autoclave treatment, as commented earlier in Part I, was given due to the fact that ζ - potential of the emulsions does not change after this treatment (Schwarz and Mehnert, 1999 and Heiati et al. 1998).

2.5 Characterization

Characterization of emulsions prepared after standardized protocol was carried out. Here in this section, the protocol of characterization will be discussed. However, the techniques used to characterize the emulsions have already been explained earlier in Part I.

2.5.1 Creaming test:

The prepared fine emulsions and sterilized samples were visually checked for any cream formed during the course of time. The sterilized samples were marked unstable if there is any visual oil content at the top of emulsions after 24 hrs of treatment. The observations of separation trends and/or creaming/flocculation as reviewed by Roland et al. (2003) and McClements (2005) were followed.

2.5.2 Particle size distribution (PSD):

Particle size distribution of any oil-in-water emulsion indicates how well oil drops are dispersed within the matrix. Generally, a smaller droplet size and narrow distribution would mean a stable emulsion and hence a long shelf life. The particle size of droplet in the emulsions was measured by both static light scattering instruments, Mastersizer 2000 (Malvern Instruments Ltd. Worcestershire, UK) and dynamic light scattering device Zetasizer Nano series –Zen 3600 (Malvern Instruments Ltd. Worcestershire. UK). The emulsions were diluted in distilled water prior to measurements to avoid multiple scattering affects. Glass beaker was used to contain the diluted sample while adding 2-5 drops per 500 ml for Mastersizer, the sample was continuously stirred and pumped to expose to the laser beam. The measurements were taken on averaging 3 readings with the help of integrated software and the particle size data was reported as either full

PSD or volume weighted mean particle diameter, $D_{4,3}$. When particle size was measured with the help of Zetasizer, definite volume of 2-5 ml was added in disposable polystyrene cuvette cells after diluting with 1-2 emulsion drops per 150 ml distilled water.

Particle size was calculated with the help of integrated software and was presented as Z-average diameter $D_{4,3}$. Measurements were repeated to confirm reproducibility and OriginLab- Pro was used to plot PSD curves after extracting the raw data from the respective software. Homogenized samples were characterized on the same day of preparation after processing. Only those sterilized samples which show stability during 24 hours were characterized for PSD.

2.5.3 Zeta potential:

The electric charge (ζ - potential) of the emulsion droplets was measured using a microelectrophoresis instrument, Zetasizer nanoseries Zen 3600 (Malvern Instruments Ltd. Worcestershire, UK). The ζ - potential was calculated from measurements of the electrophoretic mobility of the droplets in an applied oscillating electric field using laser Doppler velocimetry. Smoluchowski model was used to convert the mobility into ζ - potential automatically from the integrated software. In order to neglect the effect of concentration/dilution on the zeta potential of a sample, protocol was established in measuring zeta potential as explained by Tantra et al. (2010). The samples were diluted in their respective pH controlled solutions and were filled in disposable folded capillary cells (DTS 1060, Malvern Instruments. UK) for zeta potential measurement and repeated for different dilution levels to take only repeatable results. ζ - potential values were obtained automatically with the help of integrated software from Zetasizer. To calculate the iso- electric point (IEP), of each formulation at different pH values, raw ζ - potential data was plotted against each pH of the said formulation. Furthermore, a linear or a 1st degree exponential fit was made to calculate IEP from the ζ - potential data plotted against respective pH values. All the ζ - potential measurements were made on the same day the samples were prepared.

3. Results and Discussion

This section contains a thorough discussion on the results presented for lecithin samples dissolved in different oils with and without sodium oleate to make emulsions. Similarly, this section contains the subsequent discussion of presented results.

3.1 Fresenius Kabi commercial IV products:

Before proceeding to the results and discussions of the experimental work carried out in this part of the thesis, here in this section, the results from characterization of Fresenius Kabi commercial IV products are presented as a comparison purpose and also to have a reference product while formulating IV emulsions from different lecithin origin. Table 3.1 is an indicative of the most common Fresenius Kabi commercial products in the market utilizing egg origin lecithin. The stability of these commercial products is around two years from the date of manufacture, generally as indicated by the production/information data sheet.

Table 3.1: Characterization of Fresenius Kabi IV commercial products containing 1.2% egg lecithin and 20% oil loadings

Fresenius Kabi IV commercial products	Characterization parameters			
	pH (units)	PSD (μm)	Zeta Potential (mV)	PFAT5
Intralipid®	8.3 – 8.6	230 – 300	-53 – (-65)	0.02 – 0.025
Omegaven®	8.2 – 8.5	240 – 310	-53 – (-65)	0.02 – 0.027
SMOF Lipid®	8.3 – 8.7	250 - 320	-53 – (-65)	0.02 – 0.026

For stability study of the emulsions formulated with different lecithin types here in this section, only creaming evaluation as explained by McClements (1999) have been followed here.

3.2 Soy origin Epikuron 200 lecithin

Soy lecithin sample Epikuron 200 was dissolved in soybean and fish oil in current research. Results are presented here for the emulsions prepared with each type of lecithin with particular oil dissolution.

3.2.1 Epikuron 200 in soy oil

Epikuron lecithin samples took longer time to dissolve and a higher temperature (90 °C) was adopted to facilitate its efficient dissolution in soy oil. Longer times and in-efficient control of agitation may risk the degradation of oil at high temperatures, especially with emulsifier addition. Epikuron is a flexible yellow solid like paste with granular formation having consistency. Epikuron paste was torn apart into small granules for efficient dissolution and that strategy was successful against risking oil degradation and taking longer dissolving times.

Figure 3.1 shows the PSD curves for the emulsions containing Epikuron 200 lecithin as emulsifier at different pH values with particle size $D_{3,4}$ indicated after 5- homogenized cycles. Emulsions stabilized with this type of lecithin have no cream at their top regardless of their dissolution in oil or water. The separation trend for Epikuron sample was oil separated out at the top for high pH values while water creamed at the bottom for low pH values. Stability of emulsions prepared with Epikuron lecithin samples is presented at the end of this section. Briefly, pH 2 was the only formulation surviving autoclave treatment and stable for one week only.

All other pH values lack stability in either of the case (homogenization or autoclave treatment). In the formulations with Epikuron 200 at pH 4, formation of a gel phase was observed. It is to be noted that the gel phase was only visually detected on the rotor of Ultra-Turrax during pre-emulsion phase, while homogenization process removed the gelation. In this way, the gel phase formation during pre-emulsification had no practical limitations. Furthermore, the gel phase can be negated by changing the route of formation i.e. lecithin dissolution in water, etc. while no effect on the PSD at the same time. The particle size observed with pH 4 (gel in pre-emulsion) was having a wider distribution as compared to other pH values, indicated in Figure 3.1.

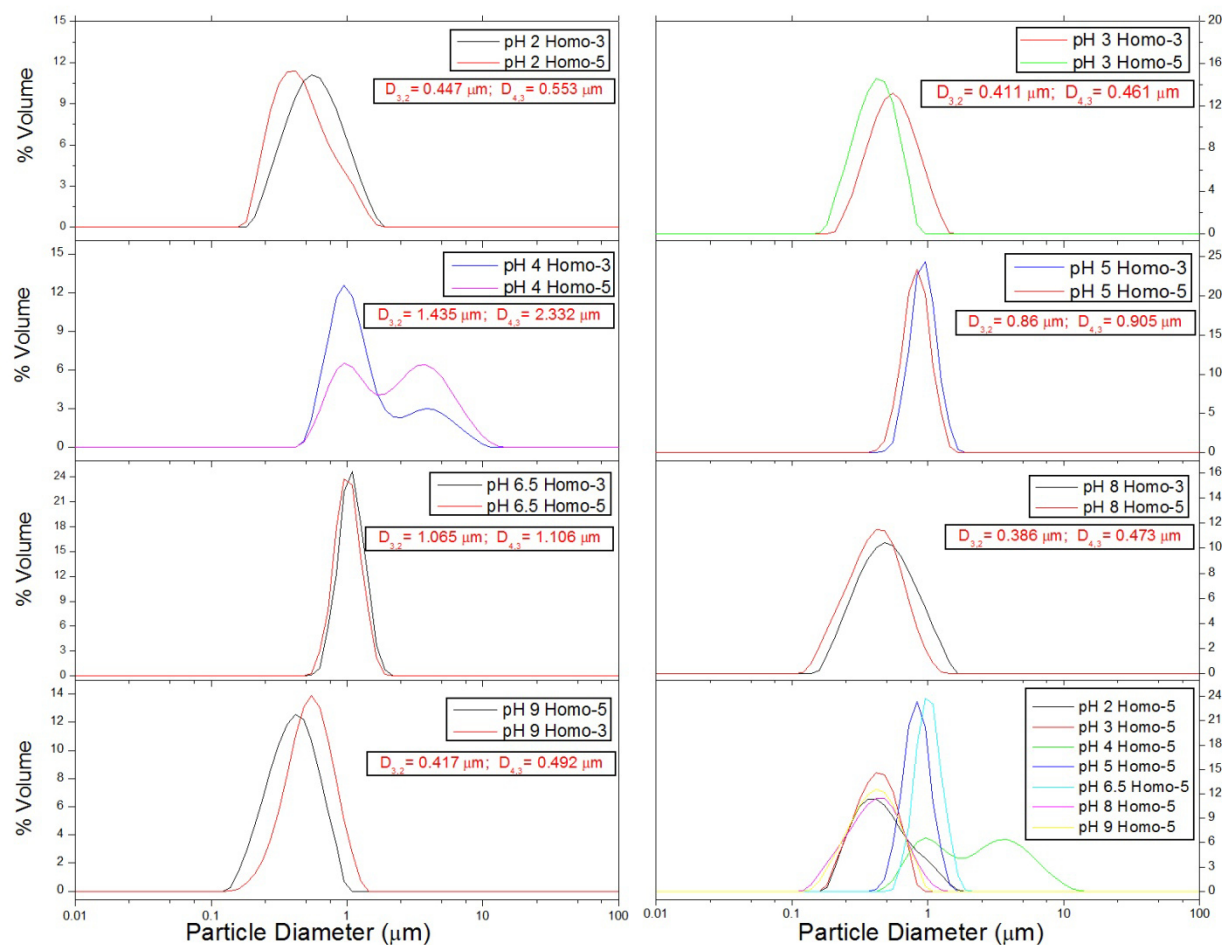


Figure 3.1: Particle size distribution curves for emulsions prepared using Epikuron 200 lecithin as emulsifier in soy oil as a function of pH values.

3.2.2 Epikuron 200 with sodium oleate

Sodium oleate added Epikuron 200 emulsion samples increased the pH of the system due to Na^+ ions, so a tight control was necessary to make emulsions at required pH values for each emulsion (pH values from 2 to 9 units). Figure 3.2 is a representation of PSD data for the co-added sodium oleate lecithin emulsions in soy oil. Clearly, smaller particle size was observed by the addition of sodium oleate as compared to the emulsions without sodium oleate. The smallest particle size of 195 nm was observed for pH 9 and unlike formulations stabilized with only Epikuron 200, sodium oleate makes pH 9 and pH 8 autoclave treated formulations stable which can also be indicated by the comparison of particle sizes of both formulations. pH 4 and pH 5 PSD values were not repeatable and these emulsions gave poor stability as well. A brief representation on the stability of these formulations is presented at the end of this section (3.1.6).

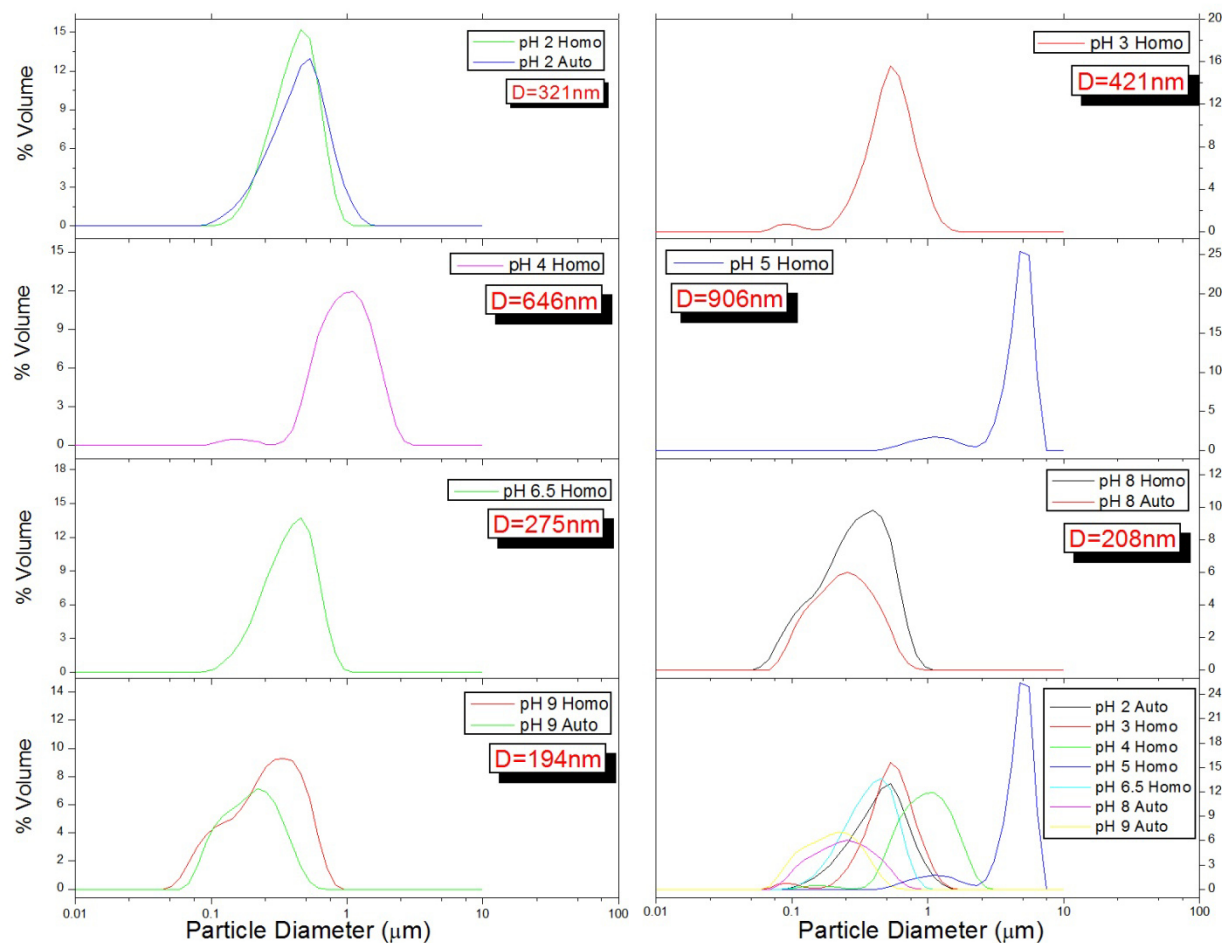


Figure 3.2: Particle size distribution curves for emulsions prepared using Epikuron 200 lecithin as emulsifier in soy oil with co-added sodium oleate as a function of pH values. (Legend: Homo is homogenized emulsions and Auto is autoclave treated formulations)

3.2.3 Epikuron 200 in fish oil concentrate

Epikuron 200 contained the maximum amount of phosphatidylcholine in its constituents (Table 2.2) equal to 92% and it is called pure lecithin in this context. For certain food applications, Epikuron 200, as a commercial product was used as pure lecithin as an appropriate choice but as far as fish oil formulations were concerned, results indicated that Epikuron 200 was not an appropriate emulsifier choice for formulations to be emulsified with appropriate stability.

Though attaining very good PSD using Epikuron 200 lecithin in the formulations prepared from fish oil, lest all the emulsions were least stable. There were incidences at higher pH values that the formulations separated completely to their respective ingredients after few minutes of preparation and water, lecithin and oil became clearly visible as separate entities in the container. Figure 3.3 shows the PSD curves for the stable formulations of fish oil only.

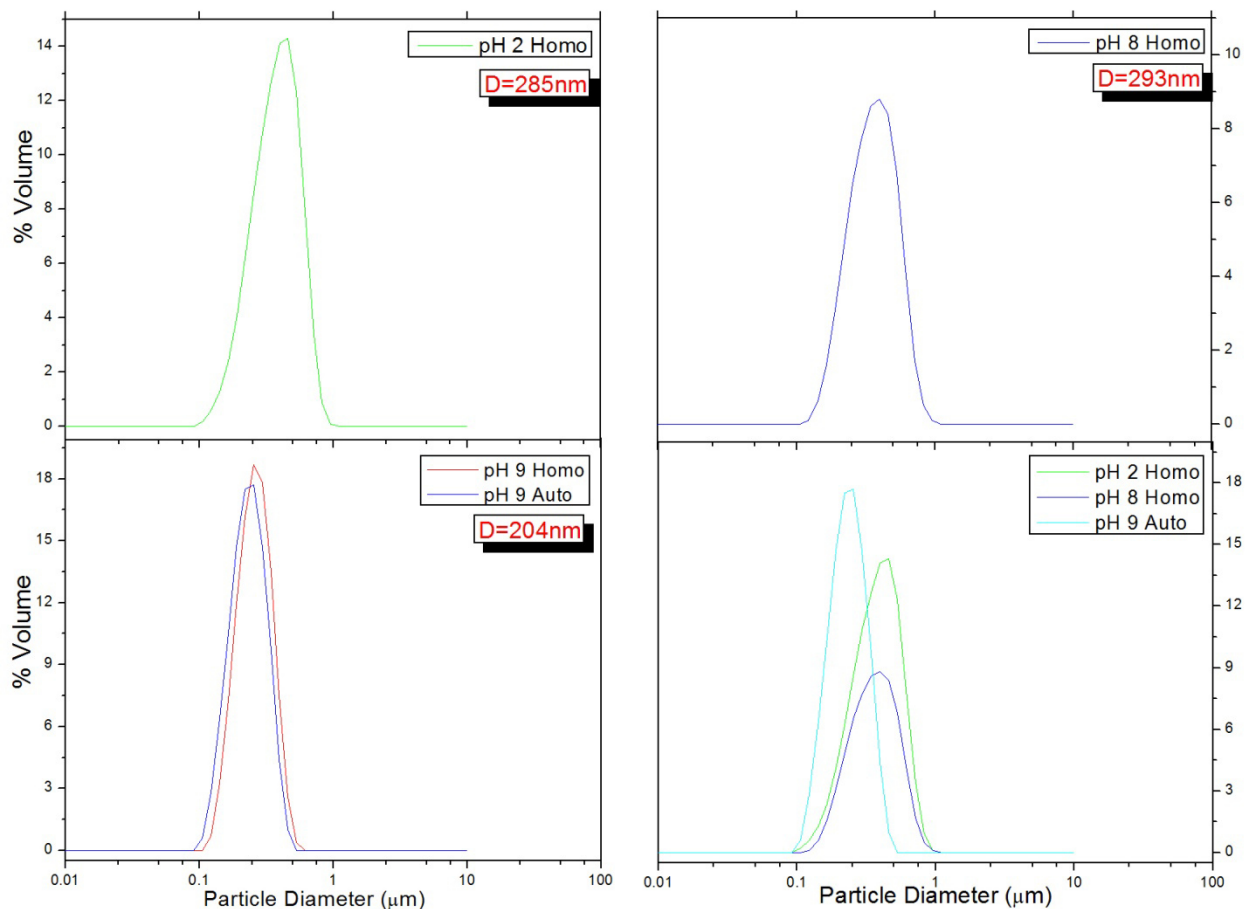


Figure 3.3: Particle size distribution curves for emulsions prepared using Epikuron 200 lecithin as emulsifier in fish oil as a function of pH values. (Legend: Home is homogenization and Auto is autoclave treated formulations).

3.2.4 Epikuron 200 with sodium oleate in fish oil concentrate

Figure 3.4 represents the PSD curves of formulations at pH 2, neutral pH and higher pH values prepared in fish oil with sodium oleate as co-emulsifier/stabilizer.

The particle size of as low as 160 nm was observed at pH 9 when sodium oleate was added as co-emulsifier. Sodium oleate basically acts as a secondary emulsifier and helped reduce particle size to a larger extent as can be seen by comparison of PSD curves with and without its addition. The particle size of lower pH values have approximately same sizes as without sodium oleate addition, the reason might be experimentally understood as lack of solubility of sodium oleate at pH values lower than neutral (acid/base dissolution for lower pH and oleic ions in sodium oleate). Sodium oleate experimentally came out of the solution as soon as the pH is lowered to neutral values. Results indicated in conclusion that either of the fact, sodium oleate was added or not, the formulations with fish oil using Epikuron 200 were least stable.

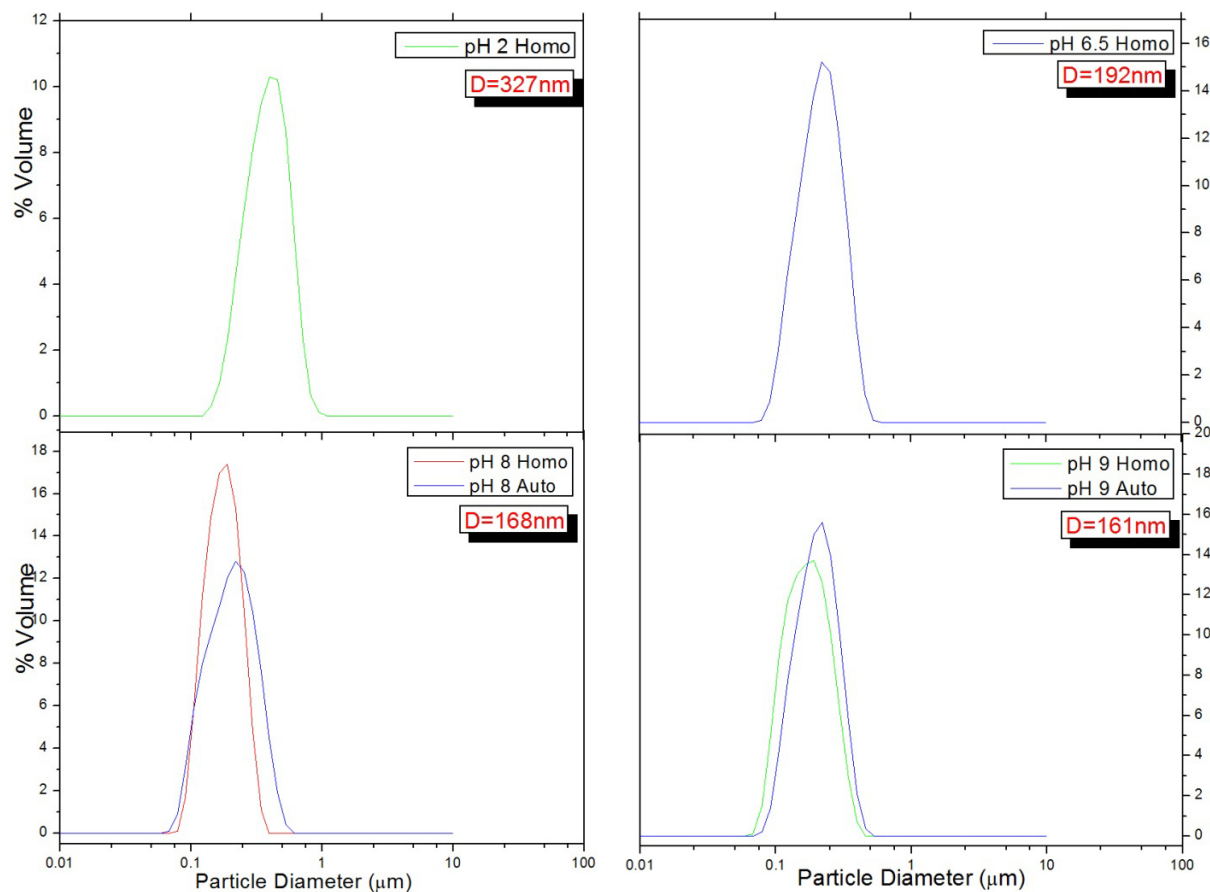


Figure 3.4: Particle size distribution curves for emulsions prepared using Epikuron 200 lecithin as emulsifier in fish oil with co-added sodium oleate as a function of pH values. (Legend: Homo is homogenization and Auto is autoclave treated samples).

3.2.5 Zeta (ζ) potential of Epikuron 200 formulations

ζ - Potential values of formulations are an indicative of the surface chemistry of droplets at that particular pH. The physical stability of the emulsions is largely determined by the composition and properties of emulsifier (in this case, lecithin) as it determines the surface chemistry. The value of this charge is quantified basically with the help of ζ - potential values (Bunjes (2005)). Zeta potential of Epikuron 200 emulsion samples were calculated with different formulations in fish and soy oil with and without sodium oleate. A typical graphical representation of ζ - potential values calculated against respective pH is indicated in Figure 3.5 as due plotted by Hsu and Nacu (2003), Chuah et al. (2009), Werling et al. (2008), Schwarz and Mehnert (1999) and Hunter (2001) in their respective research works. The plot acquired from ζ - potential values against respective pH, Hsu and Nacu (2003) have calculated point of charge reversal from their data, basically called Iso-Electric Point (IEP). In current research work, similar methodology has been followed to calculate IEP for the formulations in question, in this section, for instance.

From Figure 3.5, the IEP for Epikuron 200 formulations was calculated around 4 pH units from graph. A linear fit was made on the ζ - potential values against pH and point of charge reversal was calculated by intercept of linear fit to the zero ζ - potential values on the graph. It is to be noted that for fish oil, at low pH values, the formulations were unstable so the respective ζ - potential values for these formulations were not measured due to instability. Hence, ζ - potential values of only stable formulations are presented in Figure 3.5 for calculation of IEP.

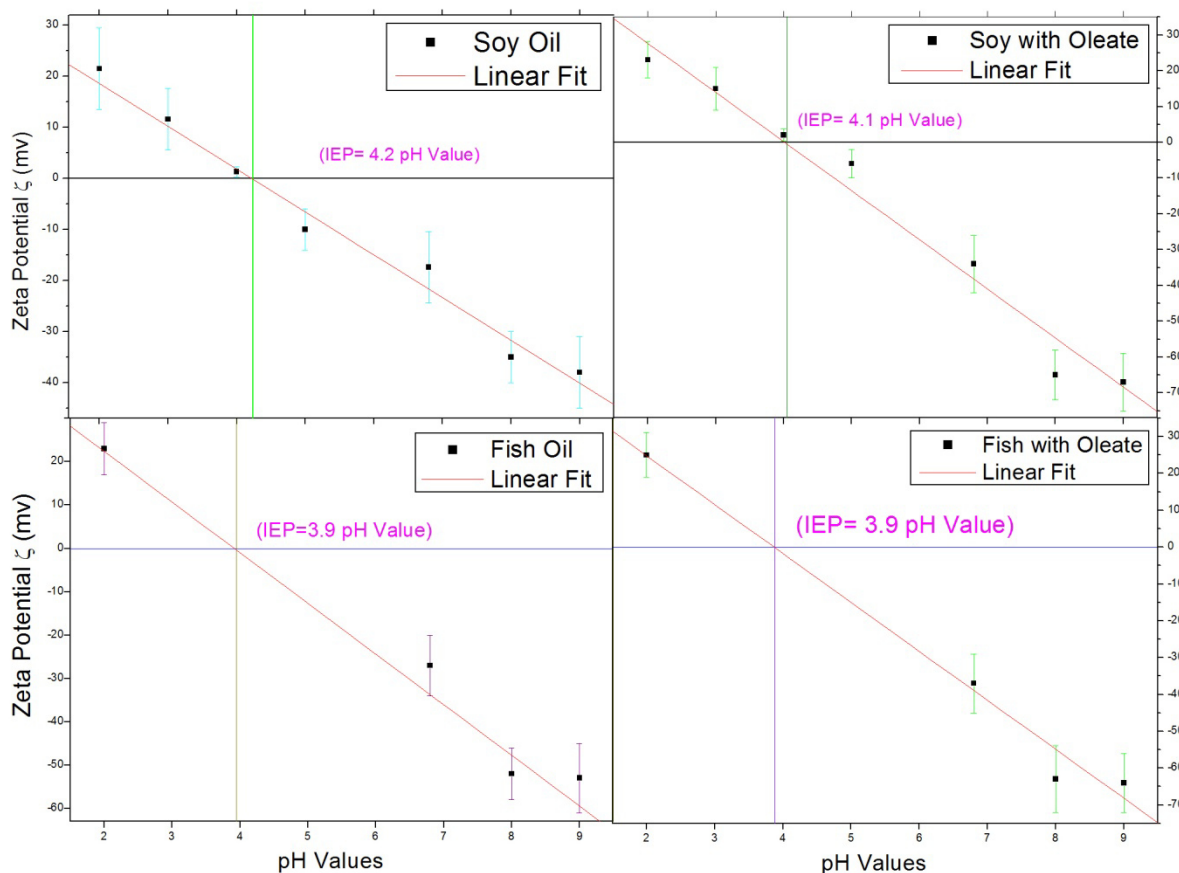


Figure 3.5: Zeta potential values of soy origin Epikuron 200 lecithin emulsion samples dissolved in soy and fish oil with and without sodium oleate as a function of pH values.

The ζ - potential values are positive at acidic pH while negative at basic pH due to respective ions in acid and base (H^+/OH^-). This drastic change in ζ - potential values is, in one way, due to the composition of lecithin, because as a lipid emulsifier it is a heterogeneous mixture of phospholipids, mainly phosphatidylcholine (PC) and phosphatidylethanolamine (PE). PC and PE are uncharged at physiological pH as indicated by Washington (1990). Epikuron 200 was used by Sjostrom and Bergenstahl (1992) and Jeong et al. (2001) directly in their research work and was considered as zwitterionic at a range of pH. Epikuron 200 also contains lyso-phosphatidylcholine-LPC (3%) with a small amount of PE too (2% max). The presence of them in purer lecithin may affect the ζ - potential values when they are ionized at higher pH as

indicated by Jeong et al. (2001) and Chuah et al. (2009). It is to be noted that PE and LPC do not present any charge at neutral pH due to their molecular structure (ionic charge balance).

Another aspect of higher absolute ζ - potential values at high pH is the balance of H^+/OH^- ions in the solution as suggested by many authors (Wiacek (2007); Mun et al. (2006); and Hsu and Nacu (2003)). Mosqueira et al. (2000) provided another view-point for high negative ζ -potential values at basic pH systems whereby studying nano-capsules, nano-emulsions and liposomes for various lecithin types (Lipoid S 100 and S 75, Epikuron 170 and 200). They assumed that the negative surface charge (-40 mV to -60 mV) imparted principally due to phosphatidic acid (PA), despite its low proportion (1-2%) in total composition of lecithin. It is therefore assumed that even small amount of PA influence the ζ - potential absolute values, however, the technical data sheet supplied by the manufacturer for this research work did not have any info on the PA percentage but this evidence is provided by Heurtault et al. (2003) as well in their research work.

Higher negative ζ - potential values did not always indicate a stable emulsion as discussed earlier in characterization section and indicated by Roland et al. (2003). Higher negative values of ζ - potential are only absolute values that along with other factors like PSD, emulsifier, oil loading, indicate stability according to DLVO theory (Kawaguchi et al. (2008); and Lieberman et al 1989)

Addition of sodium oleate further increased the absolute ζ - potential and the values became more negative for both fish oil and soy oil formulations. The obvious reason to this negative charge was due to the presence of oleate anions in formulations as discussed by Werling et al. (2008) in their phospholipid system. However, the effect of oleate anions was negligible at low pH due to insolubility of sodium oleate at acidic values or, in other words, the ionization of sodium oleate was neutralized by the addition of acid to decrease pH. Figure 3.5 shows ζ -potential curves and calculation of IEP for the respective formulations at different pH values.

3.2.6 Stability of Epikuron 200 formulations

There were only few formulations which were stable by using Epikuron 200 with soy oil. As already indicated earlier above, no formulation with fish oil survived autoclave treatment irrespective of the pH value or a co- emulsifier/stabilizer (sodium oleate) addition. The storage protocol of Epikuron after the first use of this emulsifier is very important and interestingly the phosphatidylcholine content might be prone to degradation and hydrolysis as indicated by Werling et al. (2008), forming by products which may be helpful or unhelpful during emulsification depending upon the time of use. Air tight containers were used and the emulsifier was stored at refrigeration temperatures (2 – 8°C) for repeatable, appropriate and efficient use. Figure 3.6 indicates the stability of these emulsions with different oil types and addition of sodium oleate in the formulations.

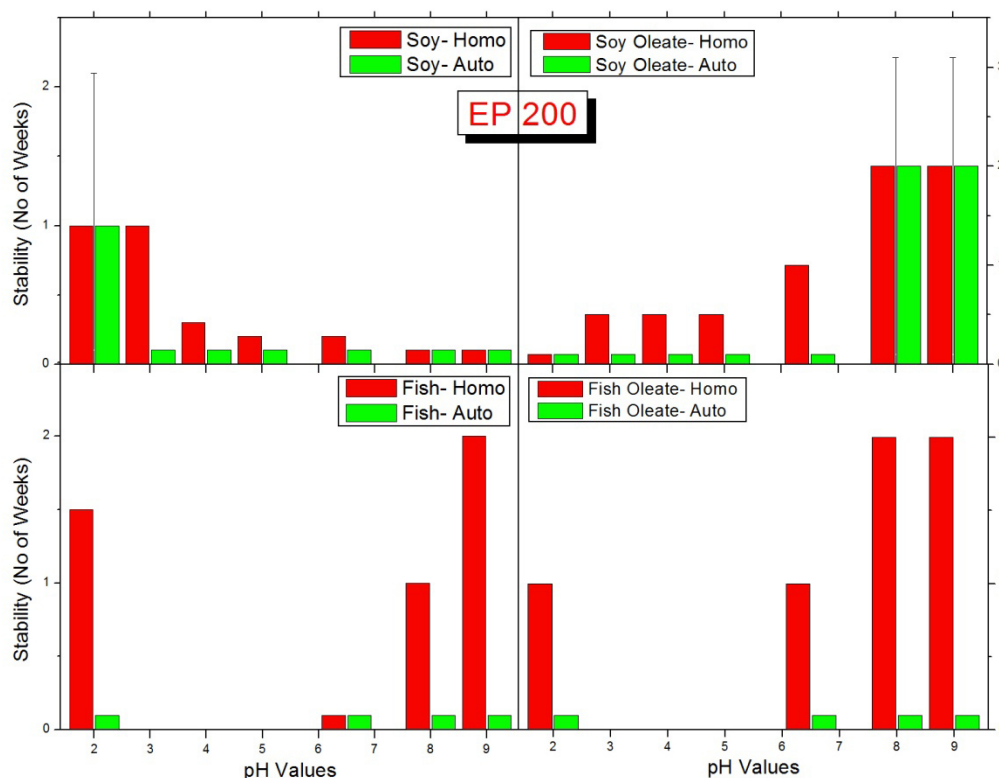


Figure 3.6: Stability of Epikuron 200 soy lecithin emulsion samples dissolved in soy and fish oil with and without co-addition of sodium oleate as a function of pH values.

Sodium oleate addition gave a life span (autoclave) of around two weeks for the soy oil emulsions at pH 8 and 9 while in contrary same emulsions were not stable. The reason behind this stability was explained by Hung et al. (2007) sharing that an addition of a hydrophilic co-emulsifier (in this case anionic sodium oleate) is required to increase the hydrophilicity of phosphatidylcholine, favoring particles interfacial curvature. The particle size obtained at that pH values was also optimum (200 nm, similar to commercial emulsions products mentioned in Table 3.1) and as indicated by Werling et al. (2008) the use of sodium oleate as a co-additive in a lipid emulsion application stabilized particle size only at elevated pH values and hence the stability too.

The experimental evidence for low stability of these formulation systems at acidic values is absolutely valid for the argument made by Fathi et al. (2012) who suggested that nano-emulsions have low stability at acidic pH. A brief graphical representation of stability in weeks for the complete system of formulations for Epikuron 200 is given in Figure 3.6 above.

3.3 Soy origin Lipoid S-75 lecithin

Emulsions prepared using Lipoid S-75 lecithin sample in fish and soy oil are discussed here in this section.

3.3.1 Lipoid S-75 in Soy oil

Emulsions prepared with Lipoid S 75 in soy oil with different pH values were formulated except than pH 2 as a gel phase was formed in pre emulsion stage when pH was controlled at 2 units. Phosphatidylcholine content, in the form of phospholipids, in Lipoid S-75 was reported to be around 70% as presented earlier in material's section, has influence on the emulsion PSD and in terms of stability and hence the ease of formulations. Amongst stable formulations, not only neutral pH and higher pH values but pH 4 and 5 were also stable. With reference to PSD curves, the minimum particle size obtained was at pH 9 in the range of 200 nm. Figure 3.7 shows PSD curves obtained by using S-75 lecithin in soy oil. PSD of pH 3, not presented in this graph below was obtained in the range of 0.4 μm .

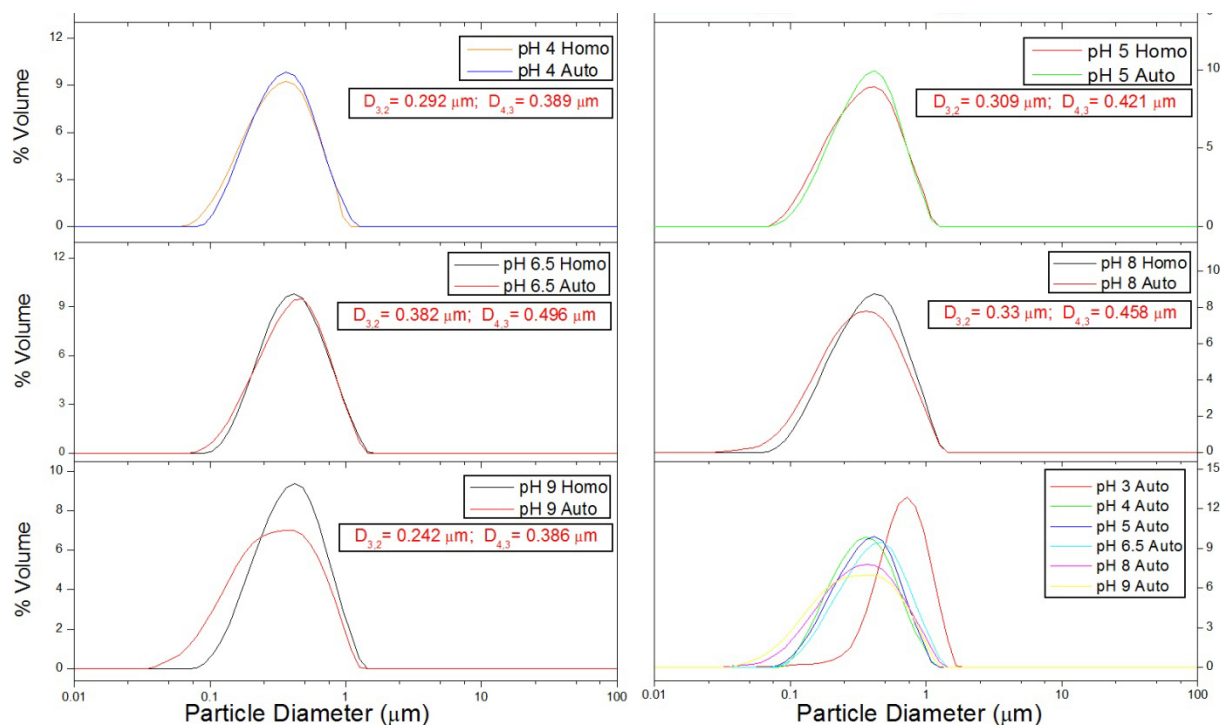


Figure 3.7: Particle size distribution curves for emulsions prepared using Lipoid S-75 lecithin as emulsifier in soy oil as a function of pH values. (Legend: Homo is homogenization while Auto is autoclave treated samples).

3.3.2 Lipoid S-75 with sodium oleate in soy oil

There was no significant difference in the formulation stability when sodium oleate was formulated with S-75 lecithin samples as compared to formulations without its edition. The minimum particle size attained was close to 200 nm as indicated in the PSD curves in Figure 3.8. pH 2 and 3 were not possible to formulate due to gel phase and uncontrolled pH values due to sodium oleate addition while pH 4 was extremely unstable as well contrary to the formulations without sodium oleate.

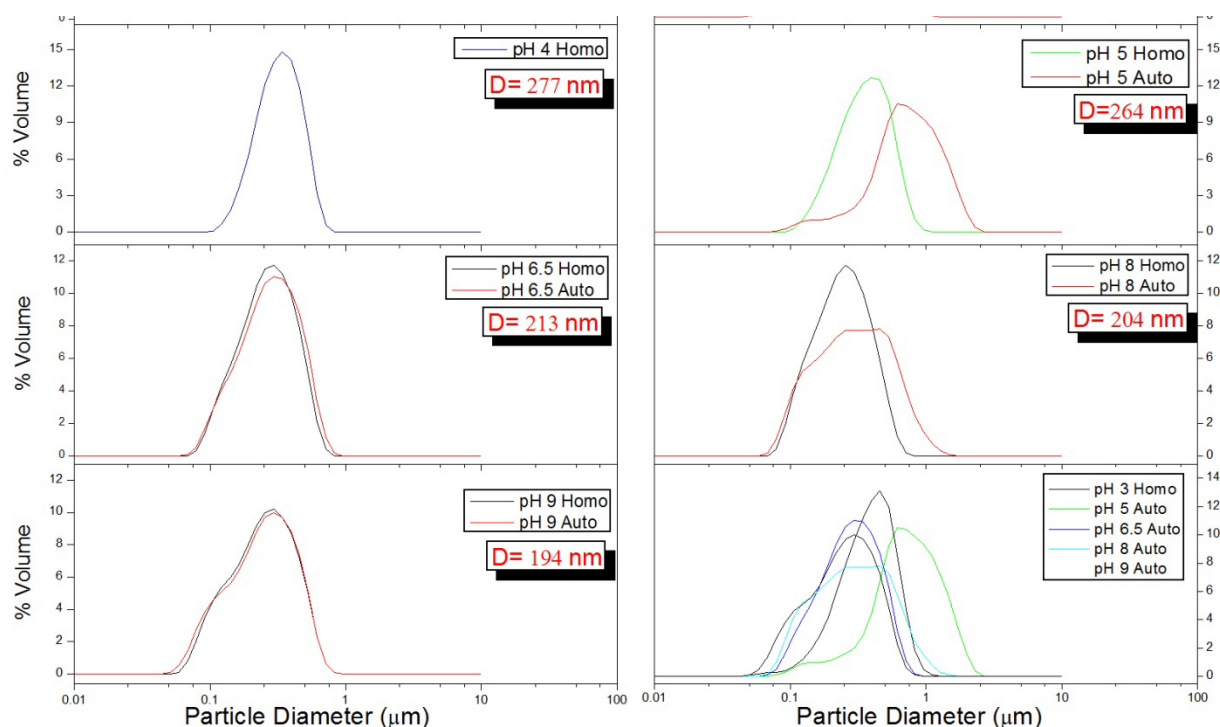


Figure 3.8: Particle size distribution curves for emulsions prepared using Lipoid S-75 lecithin as emulsifier in soy oil with sodium oleate addition as a function of pH. (Legend: Homo means homogenization while Auto is autoclave treated samples).

Particle size of both formulations with and without sodium oleate was very small and importantly, most of the formulations were stable. Here stability may be concerned with the internal contents of S-75 as indicated in Table 2 in materials section with a min 70% phosphatidylcholine. In this way, a pronounced role of an optimum amount of phosphatidylcholine in these emulsions may be the reason of stability. With reference to the phosphatidylcholine contents, Epikuron 170 with 72% phosphatidylcholine content was used to formulate emulsions in a separate study from where the unpublished experimental results indicated that emulsions were stable when this type of lecithin with said amount of phosphatidylcholine contents were used. The properties and composition of Epikuron 170 resembles with the one in question here with 70% phosphatidylcholine having similar results but

with different brand name (Cargill® for Epikuron and Lipoid® for S-75 as indicated in technical data sheet of the manufacturer). Higher quantities of phosphatidylcholine may not always serves the induction of stability as was with other Epikuron sample marked 200 with 92% phosphatidylcholine depicting poor stability for all formulations. Further discussion will be conducted when discussing ζ -potential and stability of these emulsions in section 3.3.5 and 3.3.6 with reference to the composition of lecithin (S75) and the effect of constituents on stability will be established.

3.3.3 Lipoid S-75 in fish oil concentrate

S-75 lecithin stabilized fish oil to a greater extent and to a wide number of emulsions ranging to lower pH values even. As discussed earlier in previous sub-section (3.3.2), the reason for stability may be understood as an optimum amount of phosphatidylcholine (PC) presence in S-75 (i.e. 70%). A 200 nm range particle sizes were observed for S-75 lecithin for fish oil loadings. At higher pH values, this mark went even under a 200 nm range indicating higher stability. PSD of pH 3 emulsion, not indicated in the graph below, was measured 271 nm.

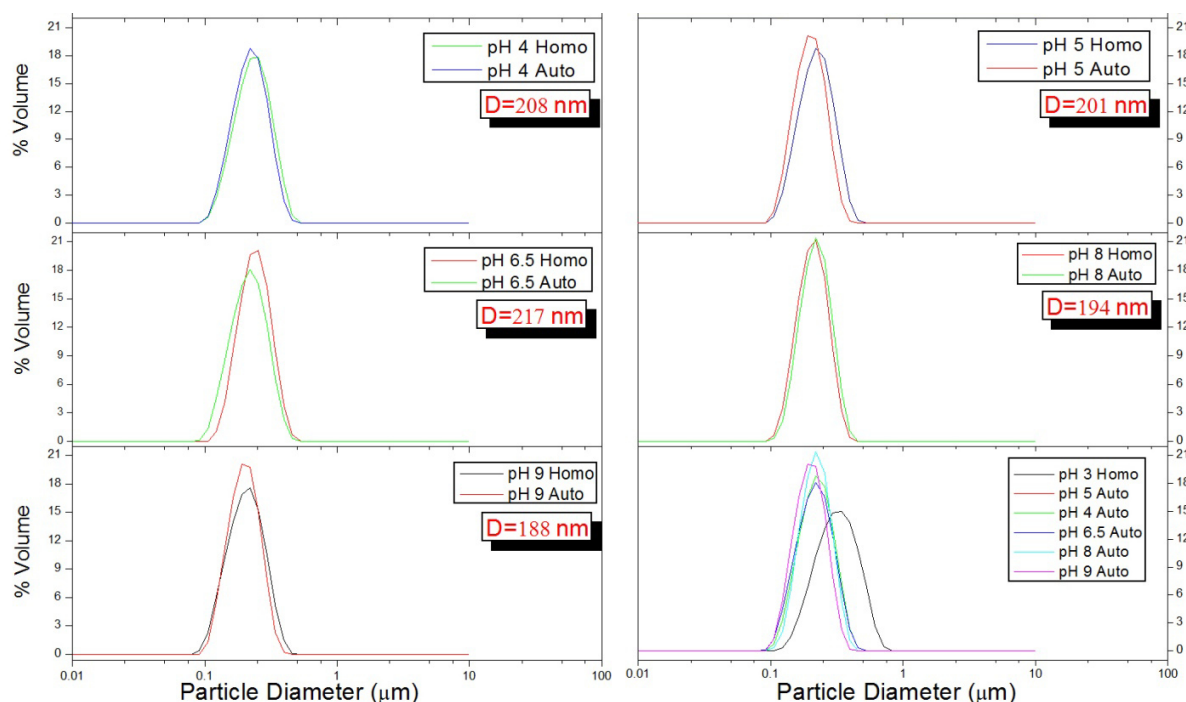


Figure 3.9: Particle size distribution curves for emulsions prepared using Lipoid S-75 lecithin as emulsifier in fish oil as a function of pH. (Legend: Homo is homogenization while Auto is autoclave treated samples).

Figure 3.9 is self-explanatory graphical presentation of the PSD curves obtained for S-75 lecithin for fish oil emulsions. A finer size distribution was attributed not only because of the PC content of S-75 lecithin but also due to the fact that the viscosity of fish oil used was less than that of soy oil and closer to water. Kong et al. (2001) discussed the viscosity relationship for continuous and

dispersed phase that if closer, lead to much smaller emulsion droplets. But at the same time the coalescence process will be faster because of easier film drainage as discussed by Netz (2004). In this way, the experimental evidence of low stable life observed in fish oil formulations even with smaller PSD may be explained by the above argument.

3.3.4 Lipoid S-75 co-added sodium oleate in fish oil concentrate

The effect of sodium oleate was also pronounced when it was added as co-emulsifier and/or stabilizer in fish oil loadings for S-75 formulations. Specifically, for pH 9, the lowest particle size was obtained in the entire formulation systems studied in the current research work. Lower particle size, especially in a 100 nm range, is also prone to flocculation due to a high surface activity and there comes the role of the electrostatic repulsive forces that must overcome this flocculation interaction due to van-der Waals forces or hydration forces as discussed by Muller and Heinemann (1994). It is currently admitted that ζ – potential values under $|30|$ mV, while optimum at $> |60|$ mV, are required for electrostatic stabilization (Heurtault et al., 2003). However, this rule cannot strictly be applied for all the systems.

Subsequent section (3.3.5) will discuss ζ - potential in greater deal when the same topic will be the subject. Briefly, Choi et al. (2011) have also indicated influence of internal attractive forces for flocculation while weakening the electrostatic repulsive forces led by the emulsifier/co-emulsifier system. They presented graphically an increase in PSD of formulation with respect to time, meaning, diffusing of the emulsifier layer (emulsifier system layer) in the aqueous phase forming flocs and reducing stability over time.

Same phenomenon was also observed in the current study when PSD was measured for autoclave samples after 24 hrs indicating an increase in particle size (making flocs from the original highly active particle surface). For example, in Figure 3.11, the particle size at pH 8 and 9 can be clearly seen to be grown (by making flocs) after autoclave treatment increasing to a 200 nm from that of 150 nm initial range when homogenized a day before and were stable (autoclave) for minimum a two weeks-time. Figure 3.11 is an indication of such type of particle transformation from very low to somewhat flocculated and/or coalesce distribution. However, the stability of emulsions was not questioned even after flocs formation. It is to be noted that the autoclave treated samples did not change their particle size along time as compared to only homogenized samples. So Figure 3.10 only represents measurements from the samples those are only homogenized before autoclave treatment. The same argument was valid for samples without sodium oleate addition when only homogenized particles changed their PSD while autoclave treated samples did not change much in comparison.

Not only Choi et al. (2011) had observed this particle-time transformation but also Chanamai et al. (2002) had observed similar variations corresponding to current data. However, the role of equipment sensitivity came into play here as they have used Master sizer 2000 for their measurements and flocculation was clearly distinguished in their measurements. However, in

current study, Zeta sizer was used with the sensitivity limitation of the equipment due to huge poly dispersivity of the measurements of droplets (oil, water, their interface or flocs) after time lag “t”. It was interesting to note that the measurement of PSD carried out with Master sizer 2000 for recently prepared emulsions were beyond its sensitive range and Master sizer 2000 was not able to detect any change in PSD along time until the particle size crossed a 200 nm mark (i.e. its active range) as mentioned in characterization section. So, a combination of measurements from Zetasizer and Master Sizer were carried out with time and presented in Figure 3.11 for fish oil with sodium oleate addition.

After 5 days, the particle size became poly-disperse and was subjected to Master sizer for its further characterization. The reason for studying only pH 9 with fish oil in presence of sodium oleate was due to the smallest achieved particle size in the studied formulation system with S-75 and a translation from a very finer to poly-disperse PSD was detectable along time (2-3 weeks until clear creaming). The fact that there might be a micro-organism growth compromise in the emulsion formulations was neglected for categorical characterization study and reported as such.

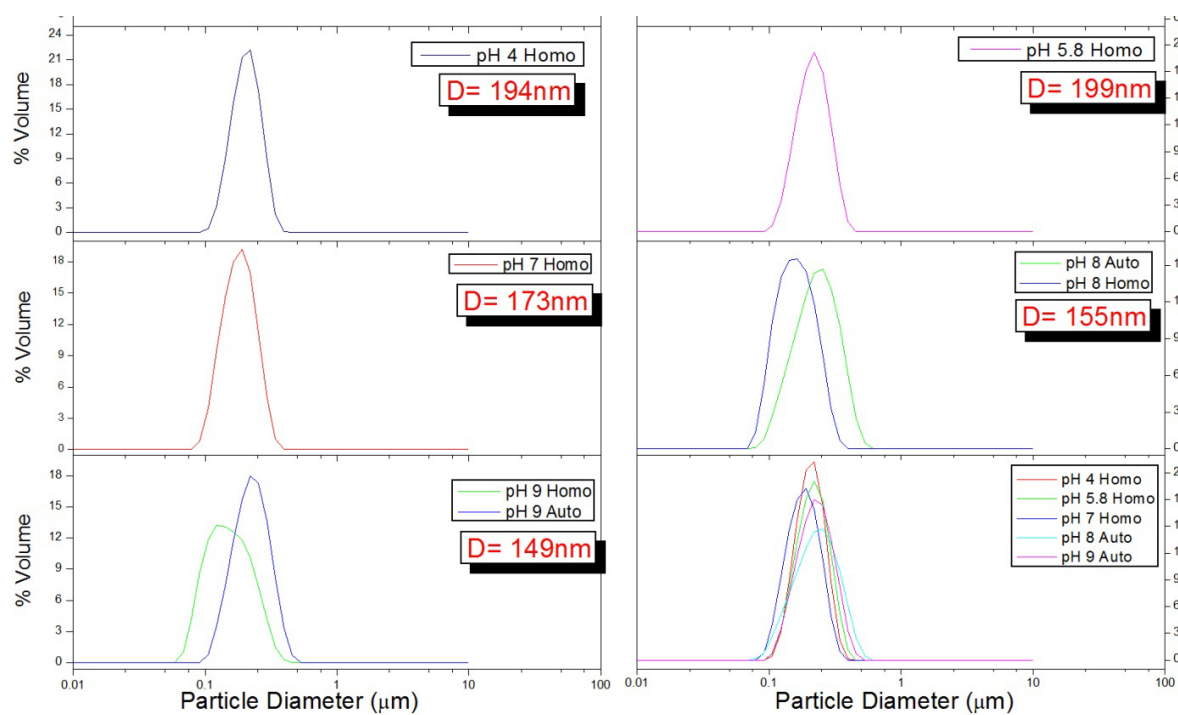


Figure 3.10: Particle size distribution curves for emulsions prepared using Lipoid S-75 lecithin as emulsifier in fish oil with sodium oleate addition as a function of pH values. (Legend: Homo is homogenized emulsions while Auto is autoclave treated samples).

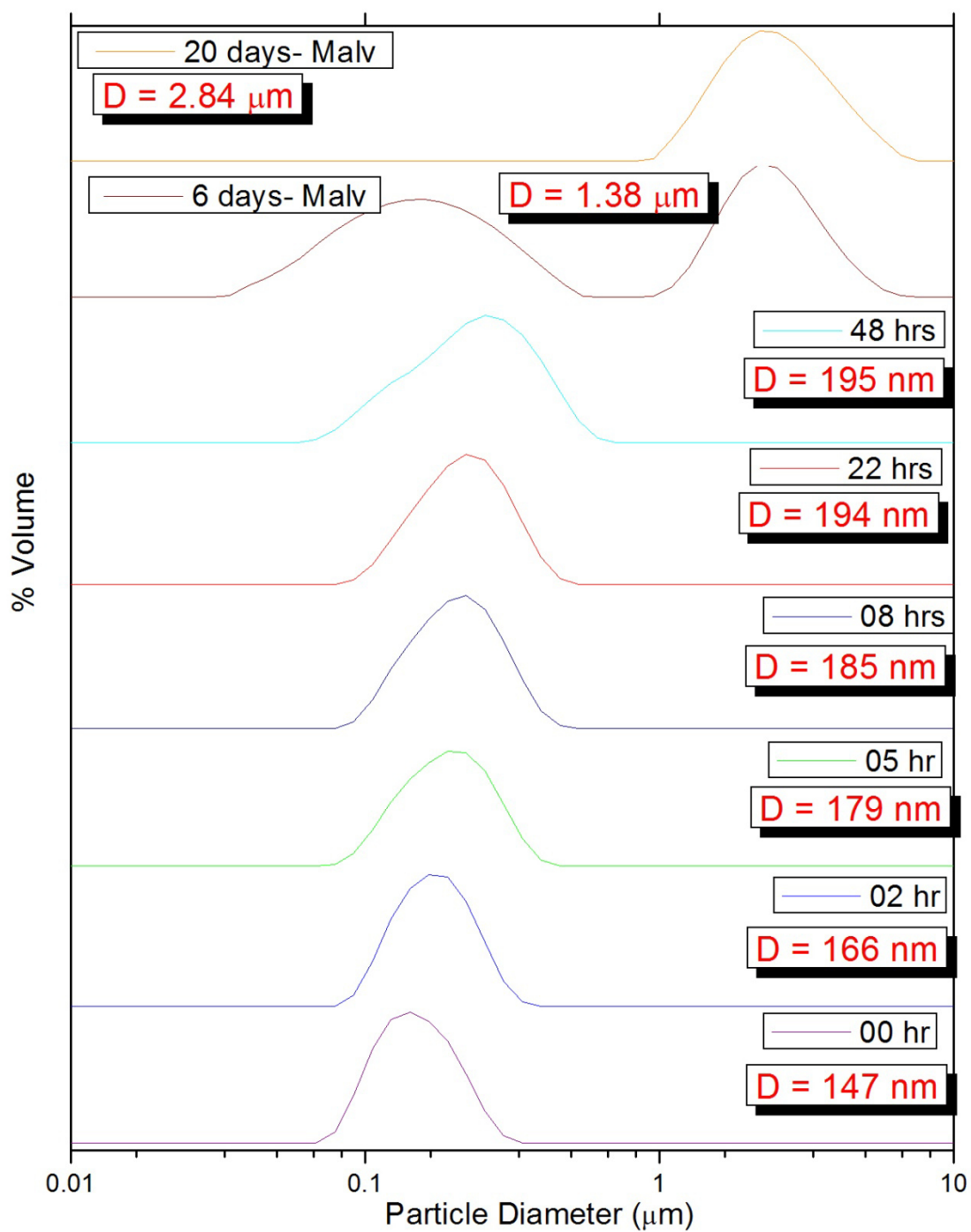


Figure 3.11: Evolution of PSD for S- 75 fish oil emulsions with sodium oleate addition at pH 9 with time.

3.3.5 Zeta potential measurements of Lipoid S-75 formulations

ζ - Potential values of systems prepared from S-75 with soy oil gave a trend that was encountered somewhat exponential along with different pH values. Furthermore, soy oil emulsions were the only formulations prepared expanded to all pH values considered in this study (i.e. 2,3,4,5,7,8 & 9 pH units). All of the values were more negative than -25 mV irrespective of the pH type and oil used, an exception to that was pH 2, which was not possible to manufacture due to immense separation (gelation) during pre-emulsion stage. In order to complete the curve for charge reversal, a separate strategy was adopted to calculate the values of ζ - potential at that pH which is presented below (basically pH adjustment during processing). The best fitting for the experimental data points was a 1st degree exponential fit to calculate the IEP for the pH systems using soy oil.

For the formulations with pH 2, the pre-emulsion was made at neutral pH and just before homogenizing; the pH was adjusted. The controlled pre-emulsions were then rapidly processed before it may form gel. The emulsions were unstable but in this way, the ζ - potential value at pH 2 was thus calculated by expanding it through the point of charge reversal and hence the IEP was calculated.

Figure 3.12 indicates the ζ - potential values of all the formulations made with S-75. The charge reversal, IEP, for soy oil systems was calculated at pH value of 2.3 units. Certain plateau in ζ - potential curve was found at a pH range between 4 and 7 units for S-75 lecithin systems. This plateau was also observed by Mosqueira et al. (2000) and reported for Epikuron 170 samples at 4 to 6 pH values which is in line with the observation in the currently studied systems. Mosqueira et al. (2000) indicated that this plateau corresponds to the ionization of all phosphatidyl groups; they reported that above this pH, the overall charge of phospholipids is negative. It is to be noted that Epikuron 170 and Lipoid S 75 are identical in their composition and origin but with different brand names (Cargill® and Lipoid®), indicated earlier.

Similarly, Hsu and Nacu (2003) gave another explanation to this plateau phenomenon that the region of flattened negative charge at high pH is a common feature of naked oil droplets and is supposedly due to a saturation in hydroxyl⁻ ions. Obviously at higher pH, particles attain positive charge so; this argument automatically does not stand anymore and ζ - potential will become more negative due to the addition of OH⁻ ions in the solution as explained earlier above (Wiacek (2007) and Mun et al. (2006).

High absolute negative ζ - potential values even at acidic pH of systems studied were due to the fact that this type of lecithin contains phosphatidylethanolamine (PE) and lyso-phosphatidylcholine (LPC) apart from phosphatidylcholine (PC) only (10% and 3% respectively as indicated in Table 2.2). PC remains zwitterionic in its form and neutral while PE remains negatively charged over the range tested as reported by Zeevi et al. (1994). Zeevi et al. studied

the ratio of PC to PE in their formulations. Similarly, Jeong et al. (2001), indicated above, stated that the emulsions stabilized with commercial phospholipids contain negative charge because of phosphatidic acid (PA) and PE. In this way, even if pH values were acidic, the ζ - potential values measured were negative and stable too as compared to other lecithin types.

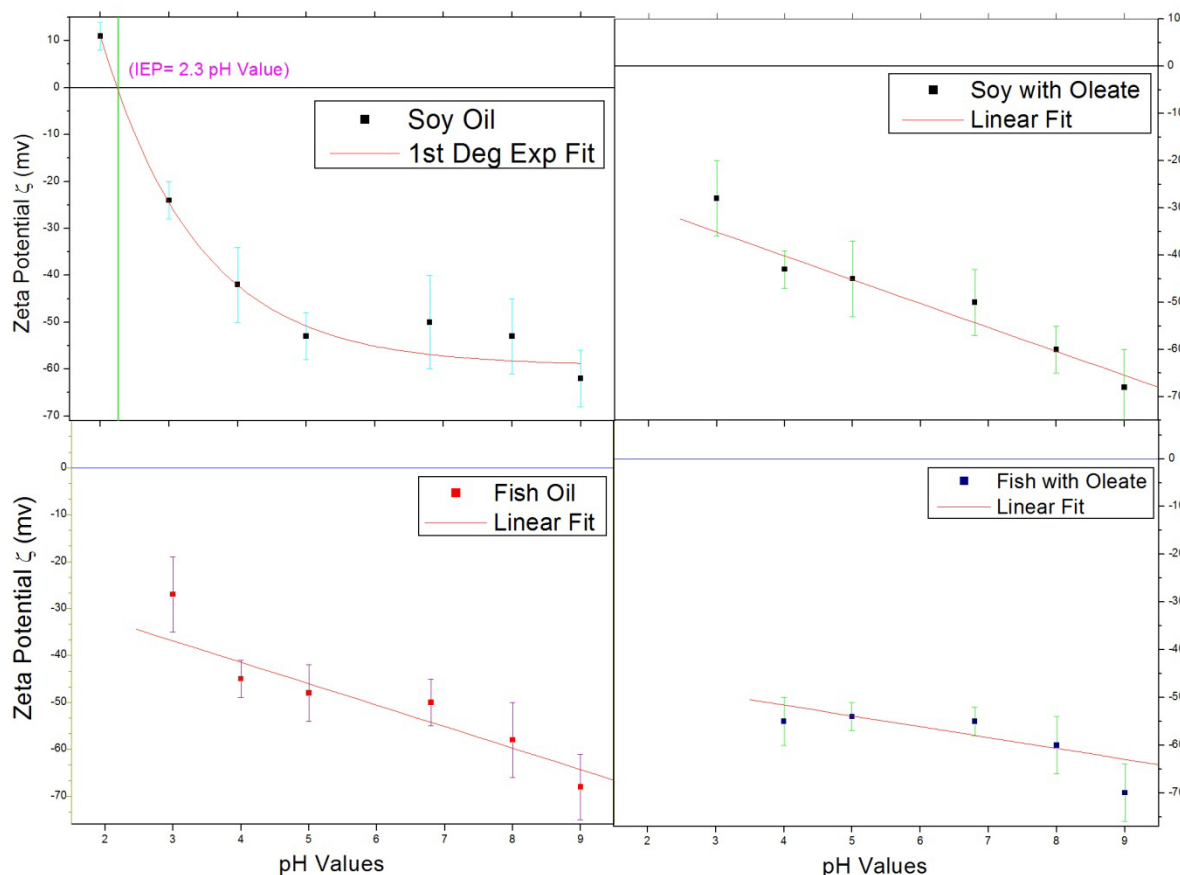


Figure 3.12: Zeta potential values of soy origin Lipoid S-75 lecithin emulsion samples dissolved in soy and fish oil with and without co-addition of sodium oleate as a function of pH values.

DLVO theory holds valid for Lipoid S-75 lecithin samples in terms of stability when only ζ -potential values were concerned, as indicated in Figure 3.12. Not only absolute values of ζ -potential were the only cause of stability but there were various factors indicated before (PSD, emulsifier, oil loading etc) that induce stability for the formulation systems discussed here.

Worthwhile to comment that all the formulations were prepared without any buffer solution and similarly the entire study was done without any buffer. It has been discussed in characterization, section 2.5, that for comparative study only, emulsions were made with buffer solution as well. So, to have this comparison, S-75 formulations containing fish oil were prepared in buffer solution with acetate buffer at acidic pH while sodium and potassium sulphate buffers at high pH values. The results indicated that the systems did not survive at any pH value for fish oil emulsions. These results were what Freitas and Muller (1999) have presented in their study that

addition of electrolytes generally reduces ζ - potential (electrostatic repulsion) in an argument that higher concentrations and higher valence of ions enhanced ζ - potential reduction and colloid/emulsion destabilization.

Aoki et al. (2005) also stated that the magnitude of ζ - potential decreases as the salt (electrolyte) concentration was increased attributed to the electrostatic screening. For that reason in this work only NaOH or HCl have been used to balance pH instead of buffer solution. Muller and Heinemann (1992) have used their systems for parenteral nutrition while they stated that the electrolyte addition lowered ζ - potential values and the values lower than -20 mV are critical for that particular use. Similarly, Schwarz and Mehnert (1999) have used S 75 in their systems without electrolyte addition. All these arguments are in line with the systems formulated in current research work in reference to controlling the pH of these systems, in general.

The purpose of a buffer is to give a tight control on the pH of a formulation. If the pH of a system is controlled by addition of ions only (OH^- or H^+ for basic and acid pH) the pH changes along time as was the case of formulations studied in current research. This phenomenon is also indicated by Zeevi et al. (1994) that the pH of emulsions decreased with time (mainly in alkaline range). However, sodium oleate, as an anionic salt, not only raised the pH of the systems but also gave a tighter control on the pH at alkaline range by keeping pH constant. As time passes and phosphatidylcholine was hydrolyzed in the system (due to the active presence of water in the formulations), the pH was reduced and ζ - potential values were affected (became more negative). This fact has been explained by Mengersen and Bunjes (2012) in their systems studied. The difference in pH from 9 to 5 units was observed significantly after 2 weeks without addition of sodium oleate while with the same formulations with sodium oleate addition, a decrease from 9 to 6 pH units was observed similarly after 3 weeks with fish oil.

The effect of buffer solution on conductivity, mobility of ions and hence on ζ - potential will be discussed in section 3.7 with formulations considered with S-45 lecithin. Formulations made with buffer solution at pH 5 and 8 with S 75 and S 45 lecithin were completely unstable and no measurement was made to calculate ζ – potential values experimentally. But it gave obvious observations that a buffer solution must affect the ζ - potential values and stability of systems as indicated by various authors referred above. Furthermore, it has already been mentioned experimentally that sodium oleate raised the pH of the systems and at low pH values; its dissolution was not possible (due to acid/basic salt interaction). In this way, the purpose of a buffer is practically denied due to sodium oleate presence and in that sense, sodium oleate emulsions at lower pH values (for fish oil systems) were not prepared, logically. However, the systems formulated with sodium oleate in fish oil have significant influence on the ζ – potential values overall and the presence of oleate anions increased the absolute ζ - potential values to more negative at basic pH as indicated by Werling et al. (2008).

3.3.6 Stability of Lipoid S-75 formulations

Lipoid S-75 prepared emulsion systems were the most optimum systems obtained in this research work in terms of stability, particle size and ζ - potential. Figure 3.13 is a brief and self-explanatory graphical representation of stability studies governed by using S-75 lecithin. The formulation seemed optimum in respect of ζ - potential, PSD and type of oil loading, etc. Sodium oleate used as a co-emulsifier/stabilizer did not influence the stability of formulations in question here significantly; rather its presence forfeited the stable systems to a lower overall stability.

The reason behind stability of these emulsions has been discussed earlier in section 3.3.2 and 3.3.5. The effect of the amount of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and another small but effective ingredient lyso-phosphatidylcholine (LPC) was crucial in interacting with oil/water layer by adsorbing on the interface to stabilize the systems and to decrease the inter-particle attractive forces by increasing the ζ – potential values. It is basically the percentage of each constituent present in lecithin that affected the final stability of the emulsions. A high quantity of phosphatidylcholine was not necessary in order to stabilize systems and rather an optimum amount is sufficient to have a positive interaction at the oil/water interface. Similarly PE and LPC has an effect on ζ - potential values and their presence here increased absolute ζ - potential values to more negative, which in terms is an absolute indicative of stability (Muller and Heinemann, 1994). This effect of composition of lecithin may be more pronounced as discussed earlier above and when other types of lecithin will be discussed in terms of ζ - potential and stability in comparison.

Also, Westesen and Siekmann (1997) who studied gel behavior of different phospholipids (S 75, S 100 and E 80) suggested that S 75 has improved stearic or electrostatic stability which is caused by the minor components of the cruder lecithin mixtures, like glycol-lipids (may up to 15% in total).

Mengersen and Bunjes (2012) also studied the same phospholipid system (1.6% concentration in emulsions) with Lipoid S 75, S 100 and S LPC for comparison purposes with the same equipment as with the current study (homogenizer and characterization equipment) but with different co-emulsifiers. They indicated that during storage the increase in the absolute ζ - potential indicate massive hydrolysis of S 75 and S 100. It is to be noted that by-products of hydrolysis of PC and PE in their respective lyso- compounds (LPC and LPE) are more effective emulsifier than PC alone due to their charging capability at elevated pH affecting ζ – potential values and hence stability. Heurtault et al. (2003) called LPC and LPE as impurities with properties of co-emulsifiers. In this way, a higher ζ - potential values were encountered for the formulations in current research along aging/stable time (for pH 8 and 9, in general) and a change in color for the emulsions with fish oil, in specific, during storage. The change in color might be because of formation of free fatty acids and lyso-phosphatidylcholine (LPC) after hydrolysis of phospholipids (mainly PC) as indicated by Mengersen and Bunjes (2012).

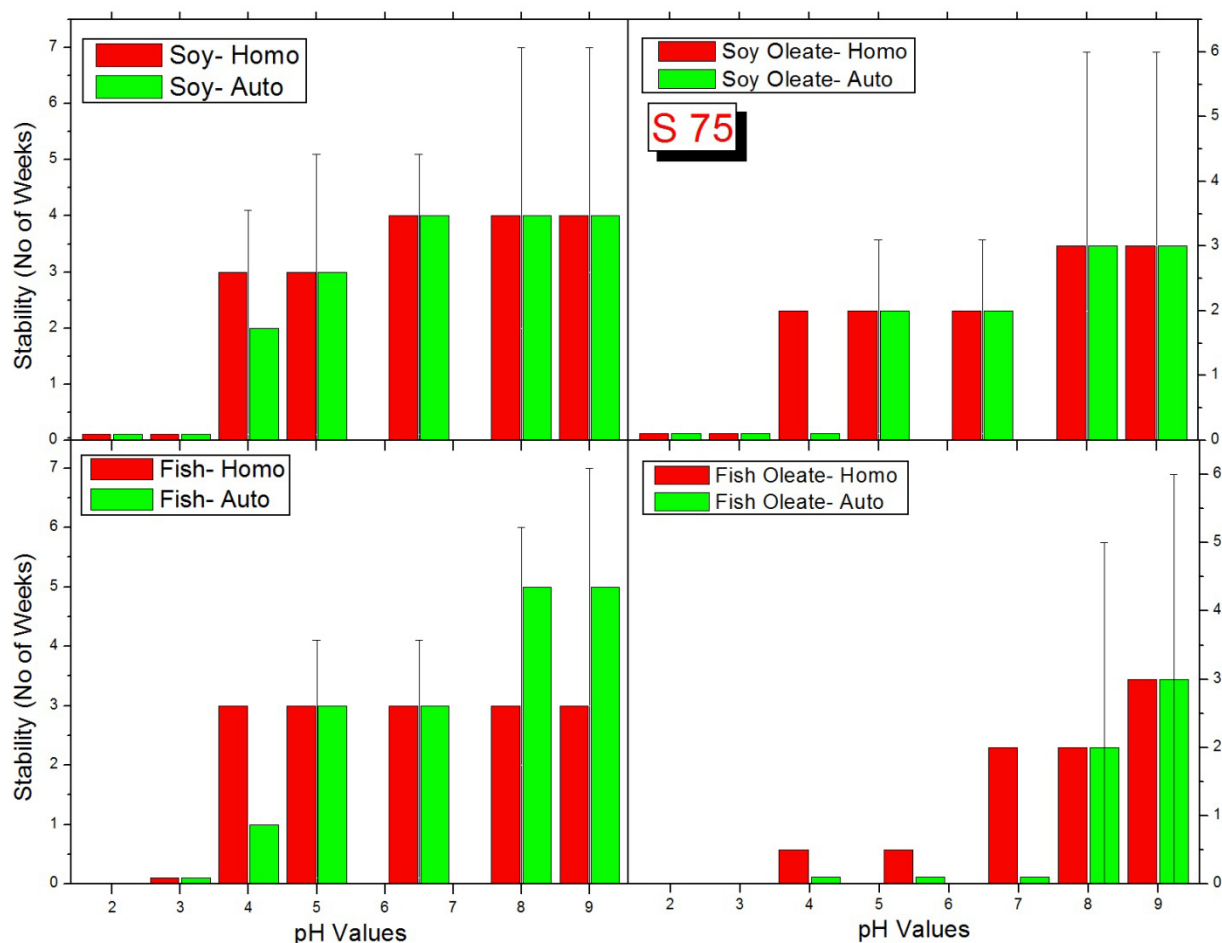


Figure 3.13: Stability of Lipoid S-75 soy lecithin emulsion samples dissolved in soy and fish oil with and without co-addition of sodium oleate as a function of pH values.

Another aspect of fish oil emulsions degradation (during time) is discussed by Serfert et al. (2009) who indicated that during the homogenization process, the droplet disruption and shearing result in large intermediate superficial area which may increase oxidation rate. In prolonged storage of emulsions, the increase in surface area determines the course of lipid oxidation in o/w emulsions as well as the interfacial thickness and its composition. That was the reason why the color of these emulsions was changed and detected after experimentation during storage.

3.4 Soy origin Lipoid S-45 lecithin

The following section will consist of discussion on samples prepared with Lipoid S-45 in soy and fish oil. The dissolution in fish oil approximately for all samples was easy while dissolving S-45 in soy oil took longer time and heating temperature was raised to 90 °C for its efficient dissolution. Emulsion prepared at pH 2, similar to S-75 sample, was very unstable for this type of lecithin as well. Change in strategy was made as described in section 3.3.5 (manipulation of pH during processing) to make pH 2 sample with soy oil, only in order to complete ζ - potential graph to calculate IEP. Briefly, pre-emulsion was made at neutral pH and before subjecting to homogenization treatment; the pH was balanced at 2 and processed. The result was the same (sample did not survive) but calculation of ζ - potential value was made possible to calculate IEP.

3.4.1 Lipoid S-45 in Soy oil

Figure 3.14 represents the PSD of soy oil emulsions prepared with S-45 lecithin. According to the composition sent by Lipoid® the alphabetical letter indicates the type/origin of lecithin (S =soy) and the number after indicates minimum percentage of phosphatidylcholine present in respective lecithin. In this way, S-45 contains basically a 45% of phosphatidylcholine. Other contents of S-45 lecithin were indicated in Table 2.2 and their effect will be discussed ahead in section 3.4.5. Particle size attained with S-45 is larger than as compared to S-75 lecithin and was in a range of 300 nm.

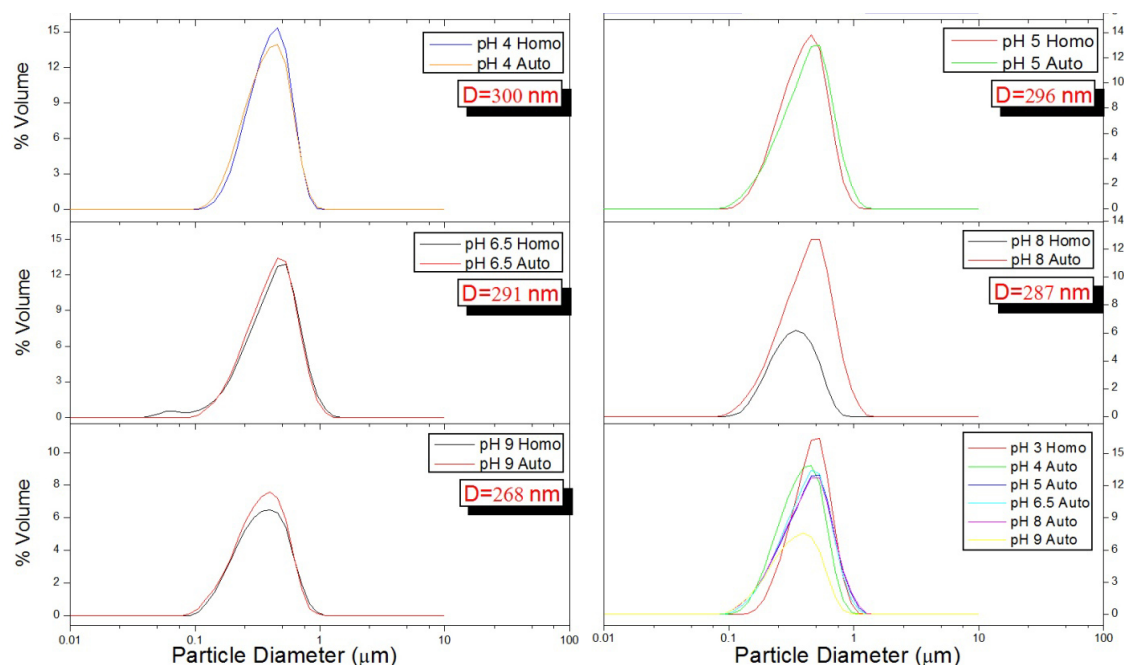


Figure 3.14: Particle size distribution curves for emulsions prepared using Lipoid S-45 lecithin as emulsifier in soy oil as a function of pH values. (Legend: Homo is homogenization while Auto is autoclave treated samples).

The autoclave treated samples with soy oil made from S 45 lecithin were stable at a range of pH values. This might be because of the composition of S 45 (Table 2.2) containing higher amount of phosphatidylethanolamine (PE) and lyso-phosphatidylcholine (LPC), which were considered co-emulsifiers by Heurtault et al. (2003) as indicated and discussed earlier above in section 3.3.6. PSD of pH 3 which was measured as 350 nm is not presented in the graph above (Figure 3.14)

3.4.2 Lipoid S-45 with sodium oleate in soy oil

Approximately same stability was found when sodium oleate was added in the emulsion samples with soy oil made from S 45 lecithin. However, as mentioned in materials and methods section, sodium oleate came out of solution/water at acidic pH so, although emulsions were successfully formulated at lower pH yet the effect of sodium oleate at lower pH has practically no impact (acidic pH neglecting effect of sodium oleate to stabilize at higher pH values). That was the reason why there is not much difference in the stability of emulsions prepared without its addition as compared to the emulsions prepared with its addition. Change in particle size was also evident when sodium oleate was added. PSD change at lower pH was not significant while at higher pH, less than 200 nm size was attained with the help of its addition that originally was in the range of 300 nm without it. Figure 3.15 is an indicative of PSD curves obtained by characterizing sodium oleate added formulations with S-45 lecithin.

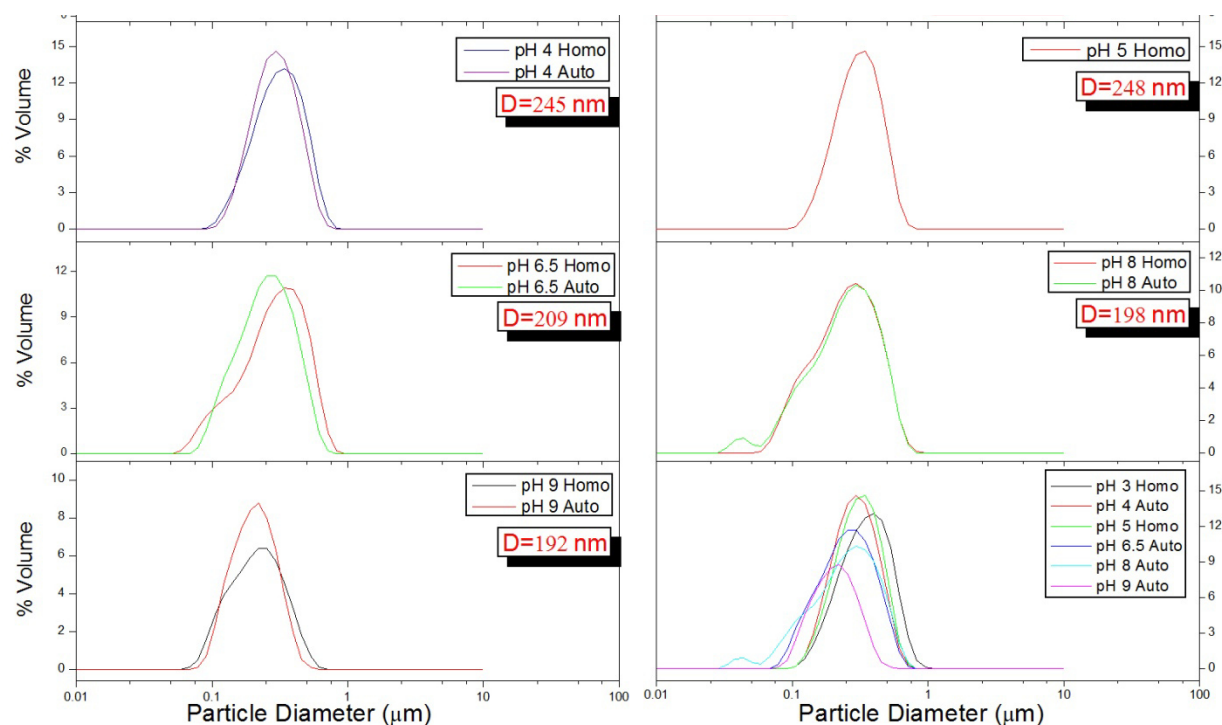


Figure 3.15: Particle size distribution curves for emulsions prepared using Lipoid S-45 lecithin as emulsifier in soy oil with sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated sample).

The stability of S 45 emulsions with sodium oleate addition at various pH values were practically because of S 45 composition and not due to sodium oleate addition itself, but the reduction in particle size is a clear indication of its effect at higher pH values. PSD of pH 3 was calculated as 276 nm while not presented in the graph of Figure 3.15

3.4.3 Lipoid S-45 in fish oil concentrate

Like S 75, samples prepared with S 45 in fish oil also showed good stability at various pH values and a minimum particle size range under 200 nm was observed (at higher pH). The reason for reduced particle size observation when fish oil was used was already explained in section 3.3.3 concerning the viscosity of fish oil in formulations. Briefly, as suggested by Kong et al. (2001), smaller PSD will be attained if the viscosity of both water and oil phase matches. Similarly reduced stable life of fish oil formulations were argued by Netz (2004) in relation to lower particle size distribution and ease in emulsifier film drainage from the inter-phase. For fish oil emulsions, similar to soy oil using S 45, the composition of S 45 containing LE, LPC and free fatty acids influenced stability giving maximum two weeks life to the formulations at basic pH. Figure 3.16 indicates PSD measured for fish oil emulsions using S-45 emulsifier.

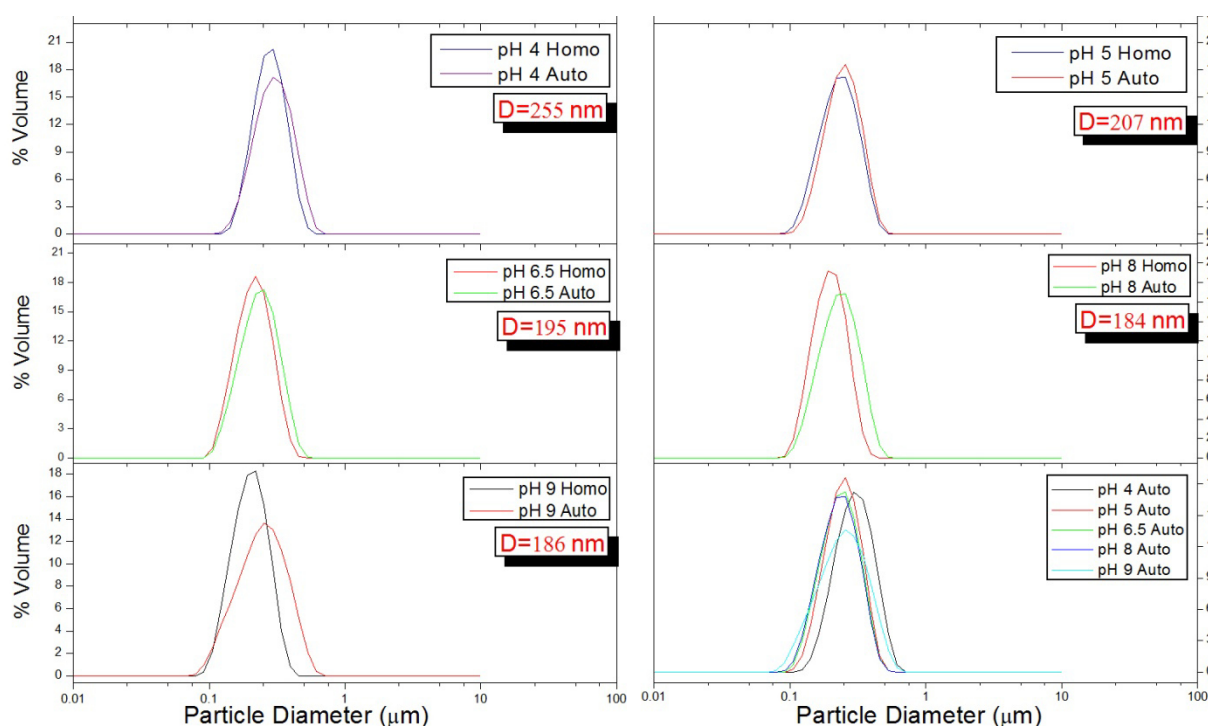


Figure 3.16: Particle size distribution curves for emulsions prepared using Lipoid S-45 lecithin as emulsifier in fish oil as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.4.4 Lipoid S-45 with sodium oleate in fish oil concentrate

Sodium oleate was an effective co-emulsifier/stabilizer in terms of reduction of particle size, especially using with fish oil whose viscosity was lower than soy oil, hence even more particle reduction was observed. However, the term stability to the formulations using sodium oleate is a relative term that has many constraints before concluding a stable life on behalf of its addition. Some of them are already discussed above in different parts of current work, such as: oil type and loading, emulsifier type and composition, pH environment with variation of time and lastly processes (and their by-products) occurring during storage. A less than 200 nm particle size was obtained by the addition of sodium oleate at higher pH, as it was discussed by Werling et al. (2008) previously.

A gel was formed at pH 5 when sodium oleate was added; it can be avoided by changing formulation strategy. All of the formulations were having a very close PSD and were almost similar when compared. Unfortunately, the closer size distribution was not helpful in imparting stability to the emulsions at lower pH in contrast to the emulsions without sodium oleate (Figure 3.20 in section 3.4.6 for Stability). PSD curves of formulations at different pH values are presented in Figure 3.17, while PSD for pH 4, not presented here had an average PSD of 200 nm.

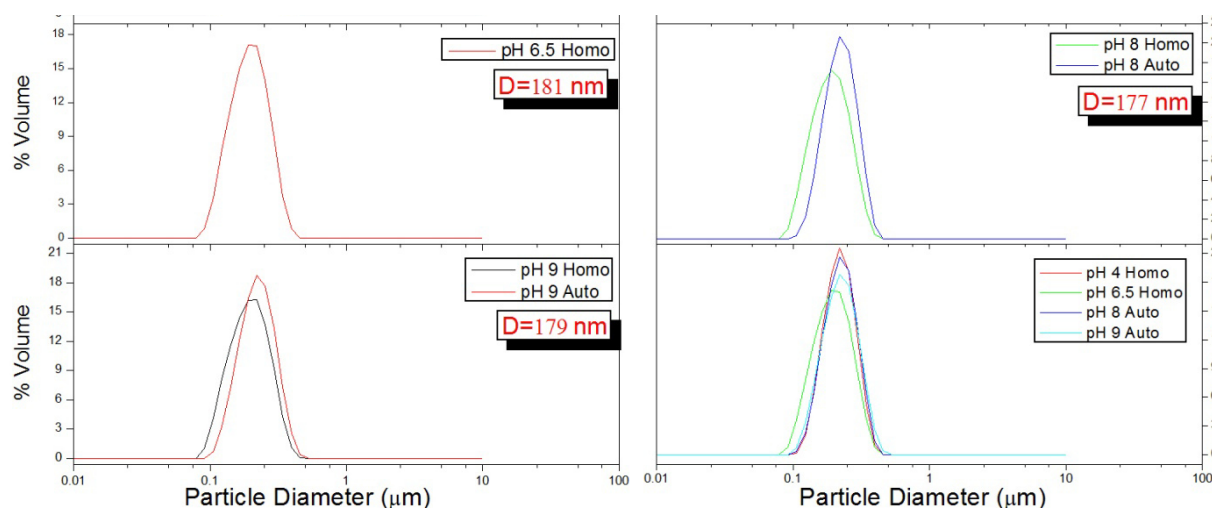


Figure 3.17: Particle size distribution curves for emulsions prepared using Lipoid S-45 lecithin as emulsifier in fish oil with sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto means autoclave treated samples).

For the evidence of the formation of gel phase with formulations at pH 5 with fish oil concentrate, a comparison of ζ - potential of S 45 was made with natural (refined) fish oil at that pH (5 units) and was calculated as -57 ~ -61 mV while surviving 1 week only.

Another useful information related to PSD evolution with respect to time was attained from sodium oleate addition with fish oil using S 45 formulations at pH 9. The idea behind choosing pH 9 with sodium oleate was what basically discussed for S 75 emulsions at same pH (Figure 3.11). Figure 3.18 indicates an evolution of PSD with respect to time for formulation at pH 9. The evolution of particle size is almost similar to what already have explained in previous section 3.3.4 with limitations of equipment as explained previously (Master sizer vs Zeta sizer). Microbial growth, however, was a parameter that was not considered in this categorical characterization procedure.

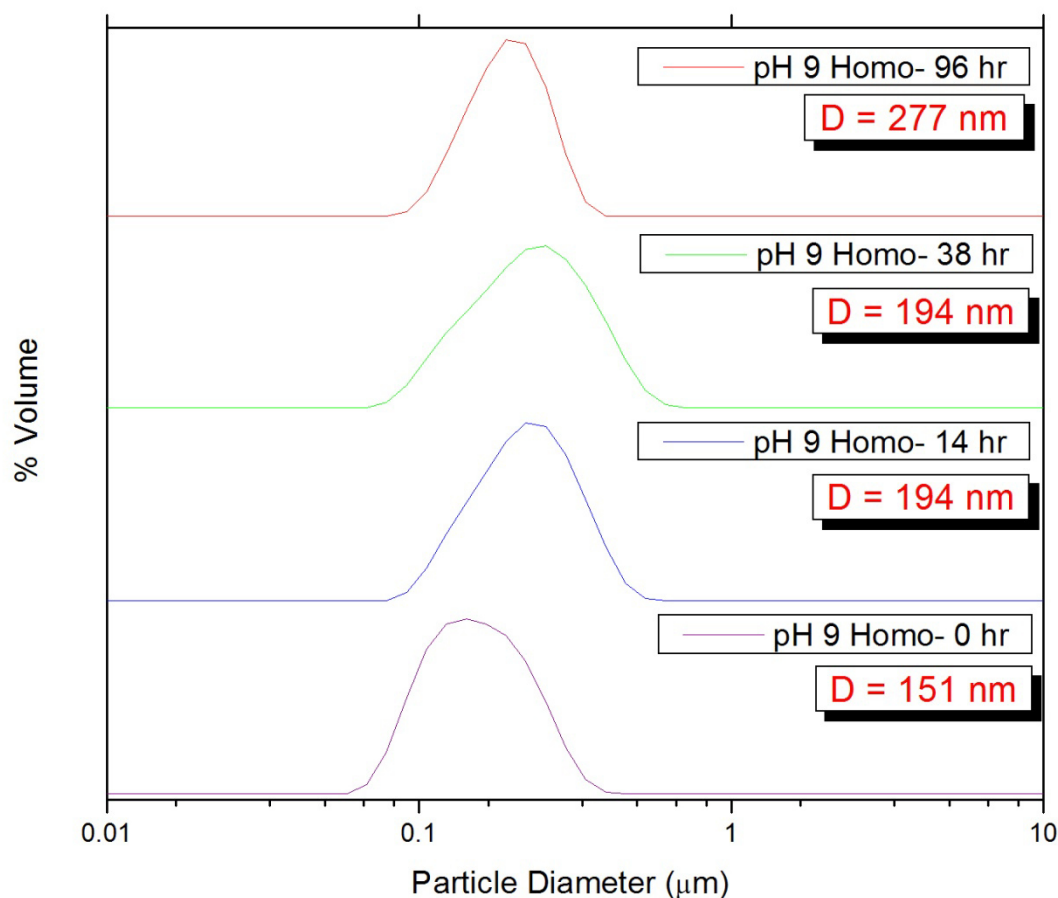


Figure 3.18: Evolution of PSD curves for S45 lecithin with sodium oleate addition with fish oil at pH 9 with respect to time. (Legend: Homo is homogenized sample)

3.4.5 Zeta potential measurements of Lipoid S-45 formulations

1st order exponential fit was made in order to calculate IEP for soy oil emulsions which was at pH value of 2.3 units. While a linear fit was made on the rest of the emulsion systems. A Plateau region was found only for soy oil and soy oil with sodium oleate addition, which was between 5 -8 and 4 -7 units respectively. For fish oil, this plateau was somewhat difficult to define;

however, for oleate addition in fish oil emulsions, due to lack of data points it can only be guessed that if it exists, might be between 4 -7 units. Physically, it seemed that DLVO theory also held valid for S 45 formulation systems according to ζ - potential and stability relationship.

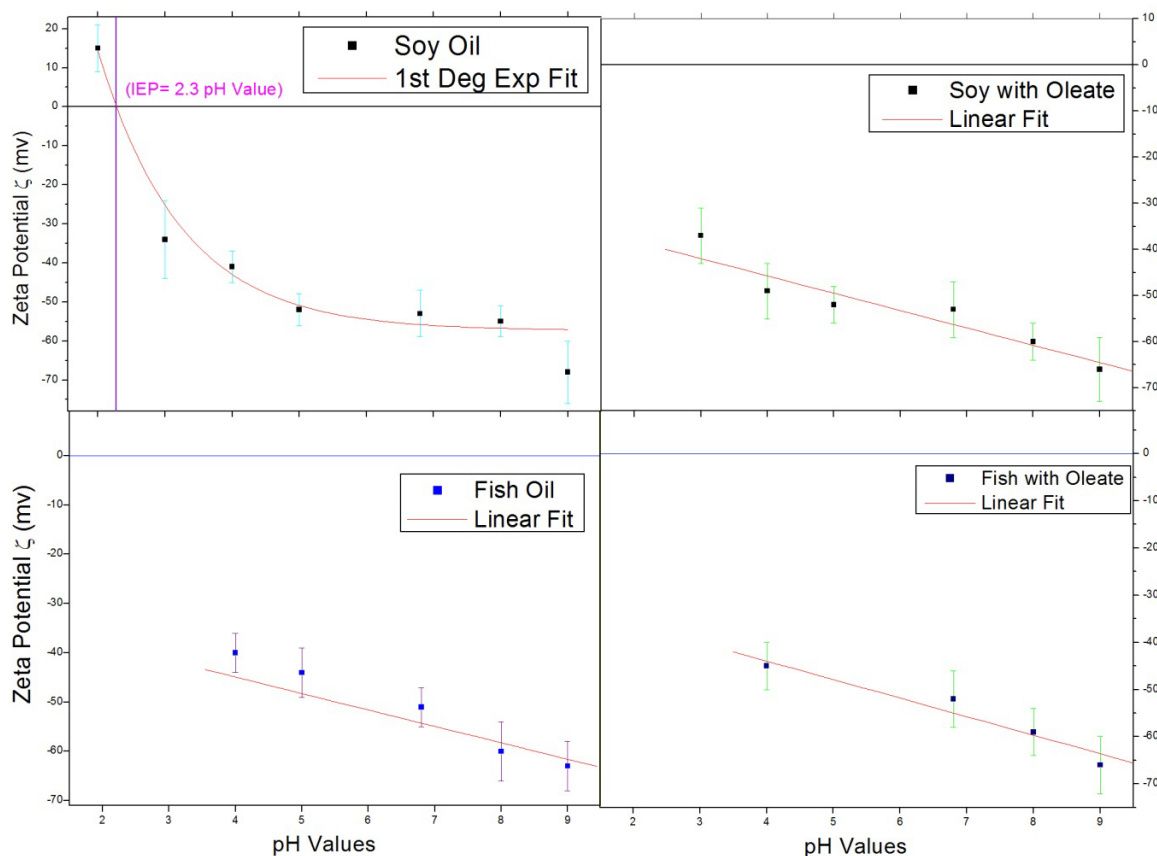


Figure 3.19: Zeta potential values of soy origin Lipoid S-45 lecithin emulsion samples dissolved in soy and fish oil with and without sodium oleate as a function of pH values.

Taking a look at Table 2.2 for composition of S 45 lecithin, we may encounter that as compared to S 75 lecithin there is an increased amount of phosphatidylethanolamine- PE (approx. twice- as compared to S 75: 18% Vs 7%) and a decreased amount of phosphatidylcholine- PC (45% as compared to 75%) while equal amount of lyso-phosphatidylcholine- LPC. It is a well-known fact that PC alone has no effect on ζ - potential due to its zwitterionic behavior, Zeevi et al. (1994) and effect of PE and LPC on ζ - potential is only pronounced at higher pH values when all phosphatidyl- groups ionizes. Same argument for negative ζ - potential values at higher pH hold valid due to OH^- ions (Jeong et al. (2001). Similarly, presence of negative charge due to phosphatidic acid (PA) in lecithin systems were referred by Alonso et al (1997) when S 45 lecithin was studied by Sonvico et al. (2006) for their nano-particle systems stabilized by chitosan. In this way, as it can be seen from the ζ - potential graphs of emulsions prepared by the two types of lecithin (Figure 3.19), there was no clear and pronounced change in ζ - potential

values and an obvious question was raised; why the stability of the two were different? The answer will be given ahead in the subsequent section where stability is discussed (section 3.4.6).

Sodium oleate gave same behavior as was previously described in section 3.3 giving more negative values and around same stability compared to without its addition. Another concern for emulsions prepared with buffer indicated in section 3.3.5 was that none of the soy oil emulsion samples survived while made at pH 8 and 5 while using buffer solution. So, unfortunately no statistical data can be presented here. But the arguments made by various authors for steric repulsions hold valid for addition of electrolytes as compared to their non-addition (Freitas and Muller (1999); Muller and Heinemann (1992) and Aoki et al. (2005)).

3.4.6 Stability of Lipoid S-45 formulations

Emulsions prepared with Lipoid S 45 were also appropriate systems in terms of stability, particle size and ζ - potential. Figure 3.20 is a brief and self-explanatory graphical representation of stability studies governed by using S 45 lecithin. Sodium oleate used as a co-emulsifier/stabilizer did not affect the stability of formulations much and its presence only influenced PSD at higher pH with lower overall stability as compared to the systems without it.

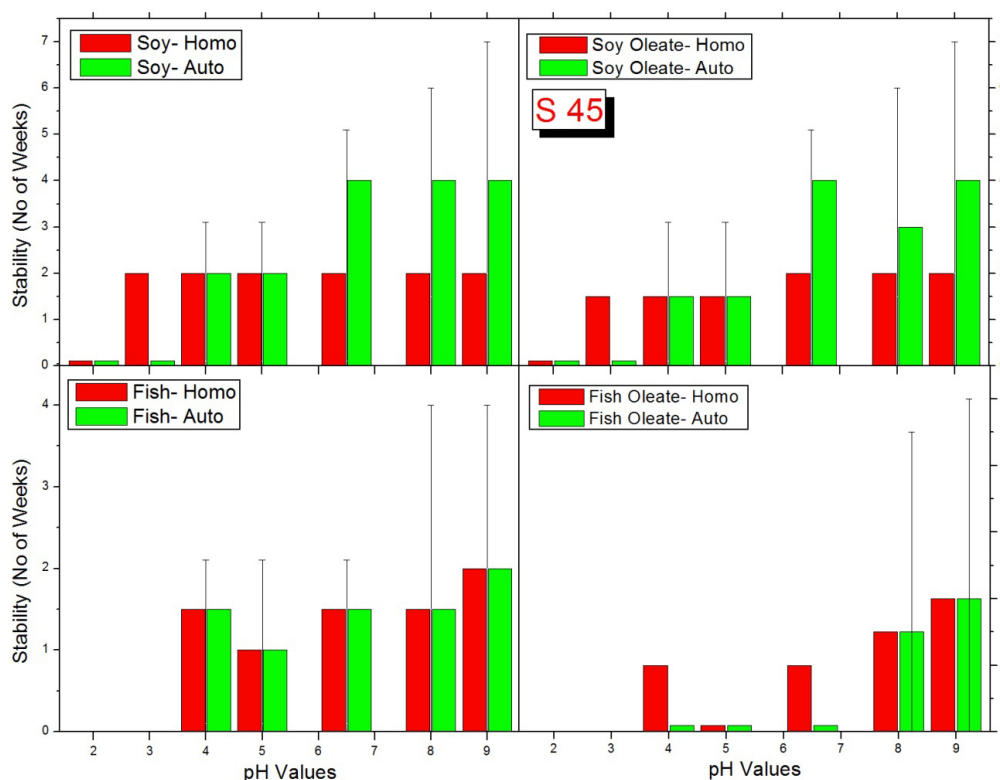


Figure 3.20: Stability of Lipoid S-45 soy lecithin emulsion samples dissolved in soy and fish oil with and without co-addition of sodium oleate as a function of pH values

The reason behind stability of these emulsions have been discussed earlier in section 3.3 and 3.4 regarding the effect of the amount of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and lyso-phosphatidylcholine (LPC).

The answer to the question raised in previous section for the difference in stability of S 45 and S 75 based emulsions with same ζ - potential values and same effects of PC, PE and LPC on ζ - values, lied in the amount of PC available for hydrolysis to produce lyso- derivatives for their subsequent use as co-emulsifier in the system during time. Clearly it can be seen that similar systems at same pH were stable for both types of lecithin, the only difference was the time of stable life which in terms can be concluded by the percentage of available PC of both lecithin S-45 and S-75 (i.e. 45% and 75% respectively). Formation of lyso- derivatives of PC (Lyso-PC) after hydrolysis during time has been referenced above in section 3.4.5. Mengersen and Bunjes (2012) and Heurtault et al. (2003) called them impurities and admire their influence on stability while Kawaguchi et al. (2008) found them more effective emulsifier than PC alone.

The change in color of fish oil emulsions was also discussed in earlier section (3.3.6) and Serfert et al. (2009) suggested phenomenon of droplet disruption and shearing, which may increase oxidation rate, during homogenization for large intermediate superficial area. For that reason during prolonged storage of emulsions, color changed due to oxidation as the emulsifier drained out of the particle interfacial film.

3.5 Egg origin Lipoid-E lecithin. E 80 S

Similar to soy origin lecithin, Lipoid E was very easy to dissolve in soy and fish oil. The physical appearance of this lecithin was in a lumpy form which was broken down before adding to the oils for its efficient dissolution. E- class of Lipoid ® is an egg derived lecithin with different phosphatidylcholine content indicated with numerical digit at the end. Lipoid E 80 S was the product used during this investigation. A chemical composition as indicated by the supplier has been indicated in materials section in Table 2.3.

3.5.1 Lipoid-E in Soy oil

Figure 3.21 shows the particle size distribution curves for the emulsions obtained by dissolving egg lecithin (Lipoid-E) in soy oil at various pH values. Particle size range for pH 2, 8 and 9 was between 0.1-1 μm while particle size of pH 3 and 4 was around 1-10 μm .

When soy oil was used with Lipoid-E, it is evident from the figure that the particle size range for emulsions with acidic pH fell between 0.5-1 μm while near neutral and at basic pH, PSD fell under 0.5 μm . Visual observations indicated change in color of the emulsions prepared with this lecithin type in soy oil at higher pH values. For those emulsion samples which survived, there was an odor observed similar to odor of the soy oil (naked particles) and raw egg lecithin. None

of the emulsion samples survived at low pH values. However, the emulsions at pH 8 and 9 were reported stable for several weeks.

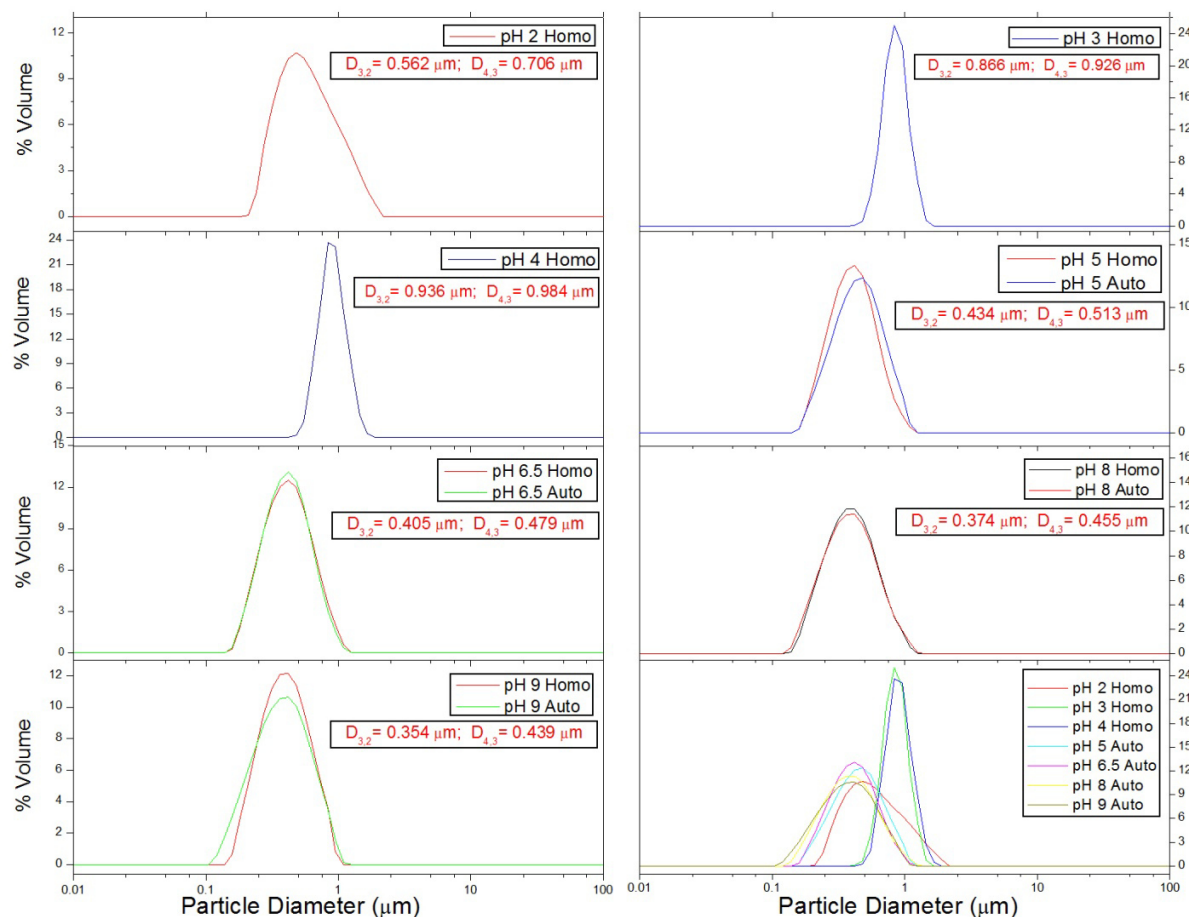


Figure 3.21: Particle size distribution curves for emulsions prepared using Lipoid-E lecithin as emulsifier in soy oil as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.5.2 Lipoid-E with sodium oleate in soy oil

Sodium oleate always influenced particle size when added in the formulations as can be seen in Figure 3.22 as compared to Figure 3.21 for the formulations without its addition. A distribution of around 200 nm was attainable with the help of its addition while it was in the range of 0.5 μm without it. Discussion was already held in earlier part of this report that sodium oleate only influenced higher pH due to its anionic nature and ionization of its oleic group at that pH, influencing only at basic pH environment as mentioned by Hung et al. (2007) and Werling et al. (2008).

A reduction in particle size was also accompanied by an increase in stability as indicated in Figure 3.26 at the end of this section for stability (3.5.6). Although samples were made at lower

pH with sodium oleate addition, it practically has no influence except for pH 2 where it showed some stability as indicated in Figure 3.26.

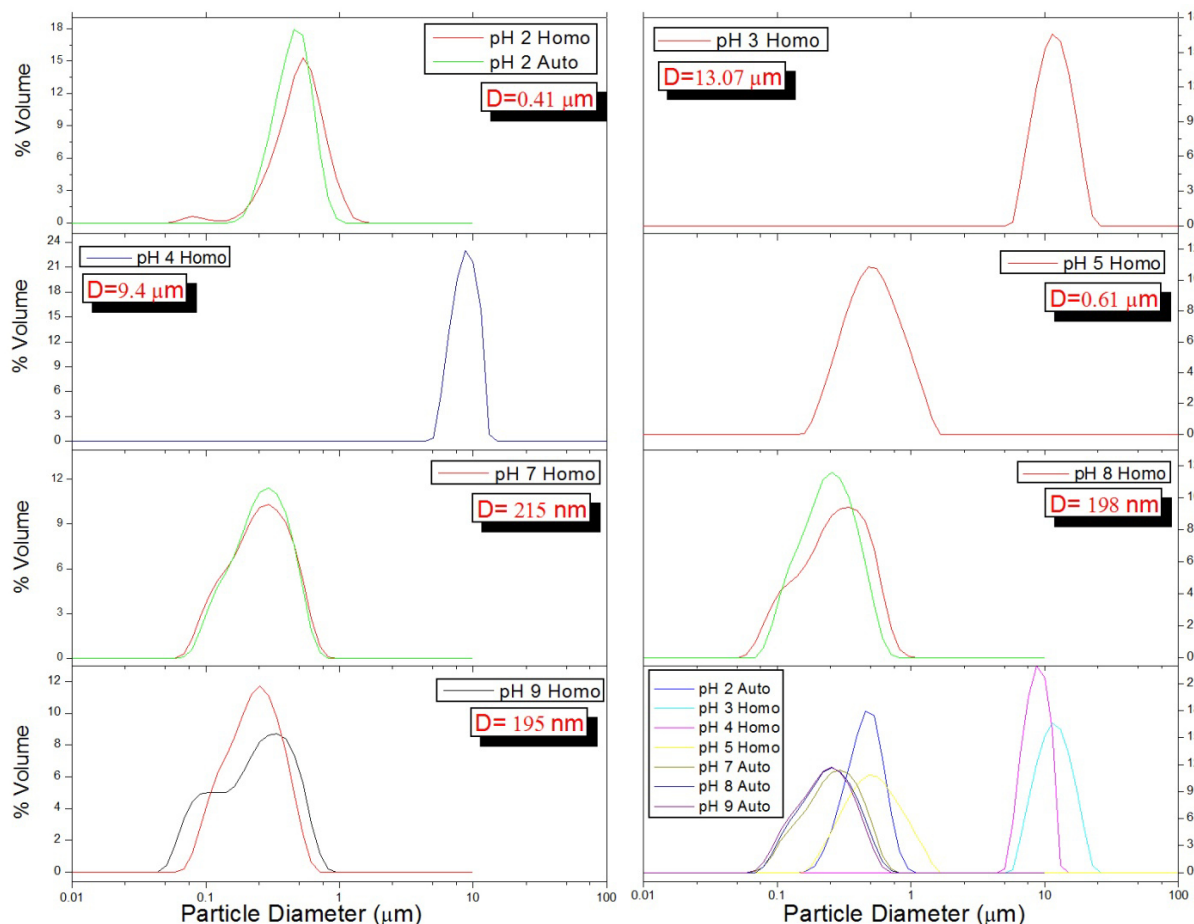


Figure 3.22: Particle size distribution curves for emulsions prepared using Lipoid-E lecithin as emulsifier in soy oil with co-added sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.5.3 Lipoid-E in fish oil concentrate

Gel formation was observed during coarse emulsion preparation when fish oil was used specifically at low pH values but was disappeared as soon as proceeded for further processing i.e. homogenization (gel structure distortion in homogenization). Figure 3.23 indicates PSD curves for Lipoid E egg lecithin emulsions using fish oil. None of the samples survived the autoclave treatment except for neutral pH value for an overnight time to at least measure the PSD. Again only higher pH emulsions gave particle size range under $0.5\ \mu\text{m}$ while acidic emulsions, as expected, were in the range of $1\ \mu\text{m}$. The color of the emulsions with fish oil gradually changed from white to off-white during aging time while giving a pungent odor. Similar observations were made in respect to the Fresenius-Kabi lecithin (PL-90) based

emulsions as well. Mean particle diameter of the emulsions prepared with fish oil using egg lecithin (Lipoid-E) is also presented in Figure 3.23 along with the PSD curves.

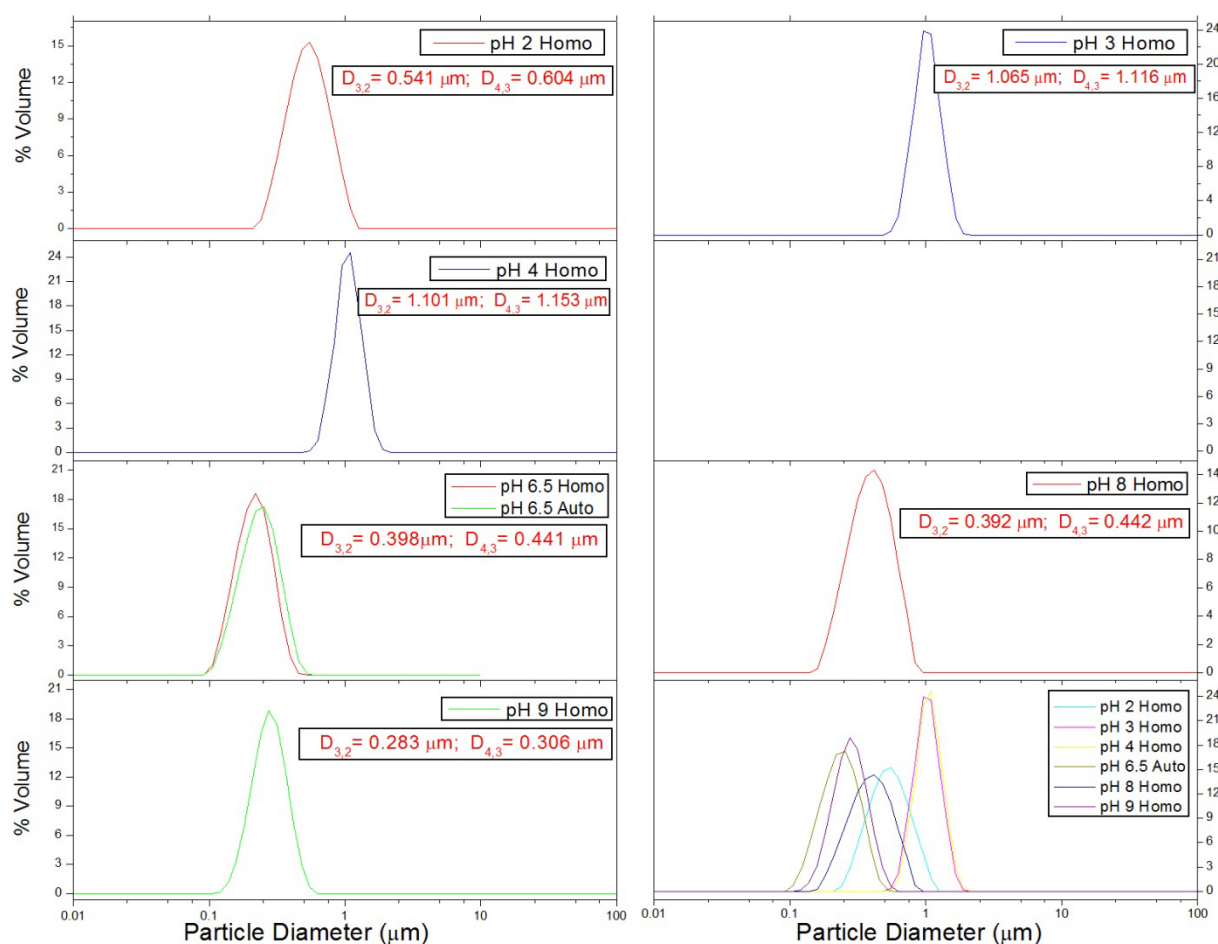


Figure 3.23: Particle size distribution curves for emulsions prepared using Lipoid-E lecithin as emulsifier in fish oil as a function of pH. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.5.4 Lipoid-E with sodium oleate in fish oil concentrate

Only neutral and basic pH emulsions were selected for sodium oleate addition when fish oil emulsions were prepared with E 80 S. Addition of sodium oleate in fish oil emulsions made particle size reduced to 200 nm range and for pH 8 and 9, around 160 nm particle size was obtained. This was not only the case with E 80 S but earlier samples with fish oil with addition of sodium oleate gave similar range particle sizes. The fact was already due explained by Kong et al. (2001) as revealed in Section 3.4.3. Figure 3.24 describes PSD for sodium oleate added fish oil emulsions using E 80 S lecithin. Unfortunately, none of the samples survived autoclave treatment using egg lecithin of E 80 S type.

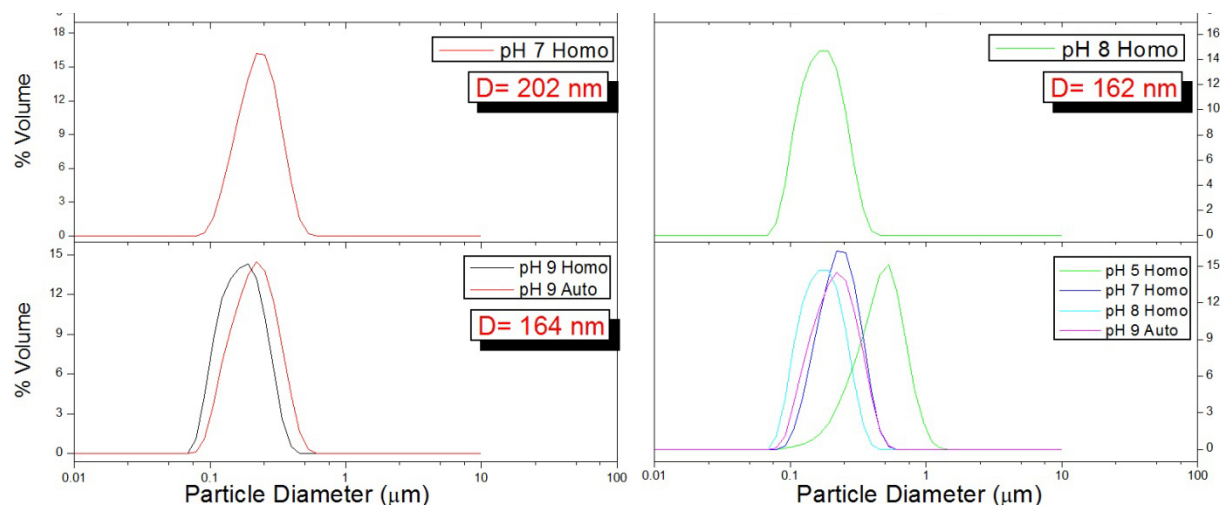


Figure 3.24: Particle size distribution curves for emulsions prepared using Lipoid-E lecithin as emulsifier in fish oil with sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.5.5 Zeta potential measurements of Lipoid-E formulations

The IEP of Lipoid E 80 S egg lecithin was calculated between 2.8 and 3.1 pH values. Different preparation route was adopted for pH 2 and 3 with fish oil due to gelation, in order to obtain its ζ - potential values. With sodium oleate and fish oil, low pH emulsions were not prepared. A 1st degree exponential fit was carried out to estimate IEP in the curve from the charge reversal point. Figure 3.25 is a graphical representation of ζ - potential values against respective pH of all the formulations.

Different authors have different opinions related to the negative zeta potential value for emulsions at high pH as mentioned earlier in sections 3.4.5 and 3.3.5 etc in this thesis. OH⁻ ions, phosphatidylethanolamine (18%), lyso-phosphatidylcholine (3%) and sphingomyelin (3%), only present in E 80 S, while compounds from hydrolysis of phosphatidylcholine, in one way or other, influenced ζ - potential values as discussed by Zeevi et al. (1994); Washington (1990); Jeong et al. (2001) and Hsu and Nacu (2003).

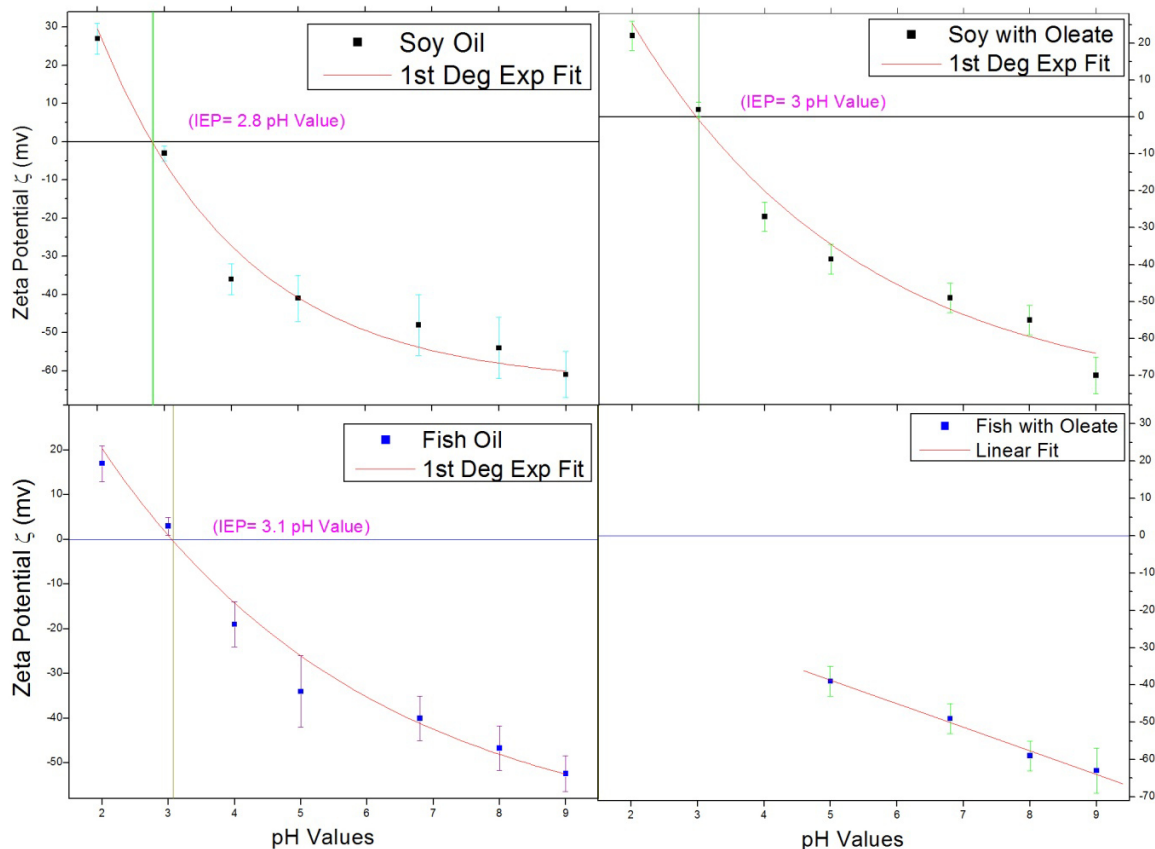


Figure 3.25: Zeta potential values of egg origin Lipoid-E lecithin emulsion samples dissolved in soy and fish oil with and without addition of sodium oleate as a function of pH values.

3.5.6 Stability of Lipoid-E formulations

Emulsions systems prepared in egg lecithin with fish oil gave a least stable life as can be seen in Figure 3.26. Those samples that survived homogenization treatment were having pungent odor and possible cause have discussed in earlier sections due to lack of oxidation resistance of fish oil particles in emulsions as soon as the drainage of emulsifier (E 80 S) carried out from the inter-phase along time, phenomenon explained by Serfert et al. (2009) and Netz (2004). It is important that emulsifier layer must remain at the droplet inter-phase because as soon as it drains out from the inter-phase, fish oil may also be prone to degradation, converting to free fatty acids (FFA), when once liberated are fatal to emulsion stability. FFA not only decreases the bulk pH and increases the magnitude of droplet but physiologically these have been shown to correlate with toxicity in animal models described by Werling et al. (2008). Hydrolysis products (lyso-derivatives and FFA) however, increases the surface ζ - potential of droplets which in terms of emulsion stability are advantageous (Rabinovich-Guilatt et al. (2005).

As soon as the partitioning of emulsifier layer at the oil-water interface occurred during time, after hydrolysis/oxidation of lecithin into its lyso- derivatives and FFA, fish oil, which is already prone to oxidation, was degraded causing not only a change in color of these emulsions but an

odor as well. This change in color for fish oil emulsions with time was only observed in lecithin with egg origin only (80 S and Lecithin from Fresenius- Kabi: PL-90).

In soy, sunflower and rapeseed origin lecithin, the change in color was not observed so it might be postulated that change in color was either related to the oxidation product of fish oil liberated in the egg lecithin emulsions and/or the interaction of these oxidation products with the hydrolyzed phosphatidylcholine content of egg lecithin.

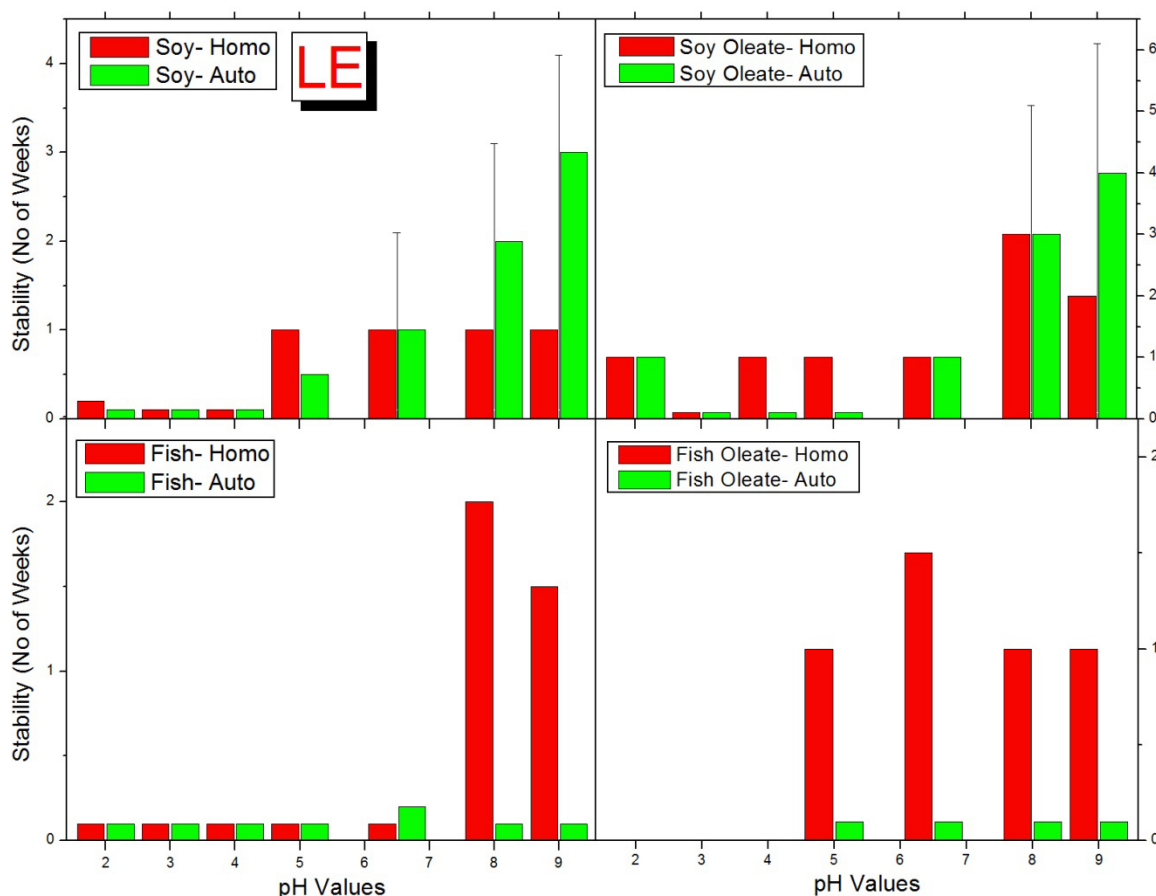


Figure 3.26: Stability of Lipoid-E egg lecithin emulsion samples dissolved in soy and fish oil with and without addition of sodium oleate as a function of pH values.

The overall low/no stability of E 80 S emulsions with fish oil may be due to higher fish oil concentration and lack of additional emulsifiers. Klinkesorn and McClements (2009) have used only 15% tuna oil with 3% lecithin and various percentages of chitosan for their formulations. Similarly, for soy emulsions, Kawaguchi et al. (2008) used 1.2% and 2.4% purified egg lecithin for 10% and 20% soy oil and studied their stability for one week time at pH values near 8 units. ζ - Potential values and stability period attained by them were similar to the ones obtained in the current work.

3.6 Egg origin Lecithin from Fresenius- Kabi: PL-90

Egg origin lecithin was supplied from Fresenius-Kabi marked as PL-90. The composition of this lecithin emulsifier (PL 90) is indicated in Table 2.3 above. Same set of experiments were conducted by using this lecithin for fish and soy oil with addition of sodium oleate. It has a powdered yellow appearance and took longer times to dissolve in soy oil and at higher temperatures (i.e. 90 °C). Fish oil dissolution was easier and faster at relatively low experimental temperatures (i.e. 55 to 60 °C).

3.6.1 PL-90 in Soy oil

For emulsions prepared by dissolving this egg lecithin in soy oil, no gel was formed at all pH values during processing. At pH values of 8 and 9 units, oil separated out initially after autoclave treatment in the form of small droplets but there was no phase separation at the top observed, however, the oil droplets indicated creaming of oil phase as discussed by McClements (1999) & (2005). Figure 3.27 indicates the PSD curves obtained after characterization of emulsions prepared with PL-90 in soy oil at different pH values.

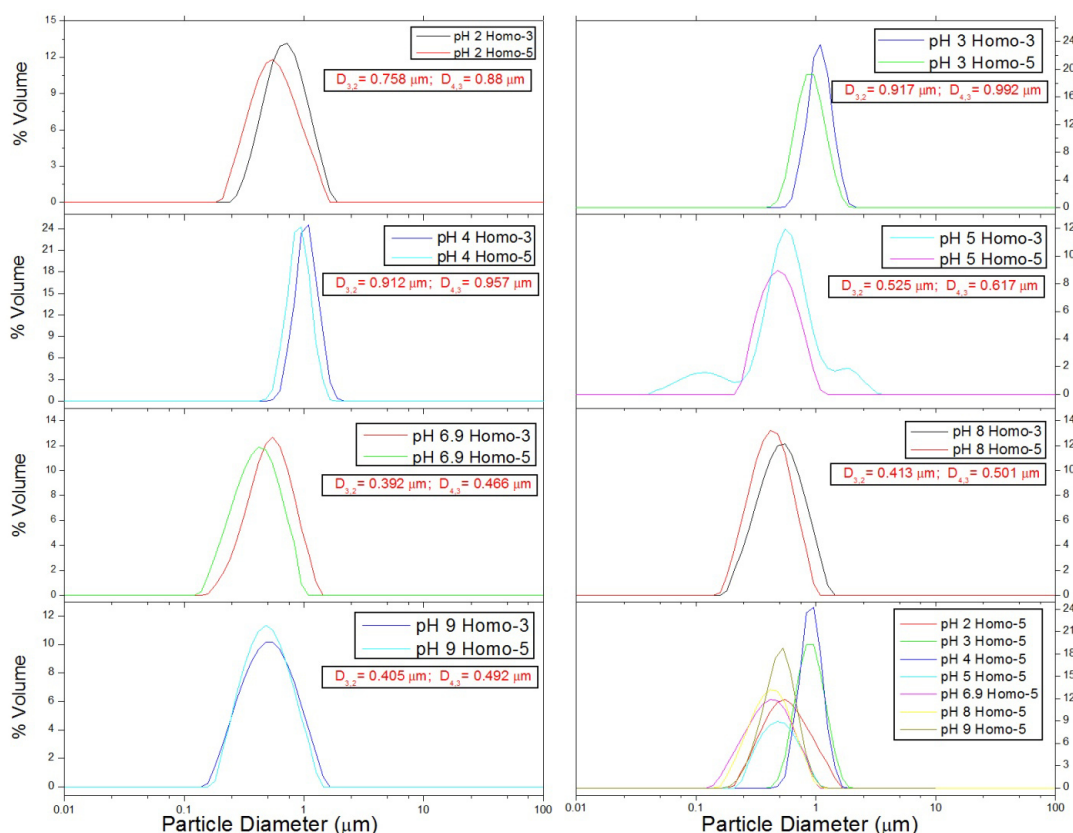


Figure 3.27: Particle size distribution curves for emulsions prepared using lecithin from Fresenius-Kabi Sweden as emulsifier in soy oil as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

There was a little effect on the color of emulsions prepared using egg lecithin dissolved in soy oil. There was an odor coming from the emulsion samples but the magnitude of odor was not as high as was with the case of emulsions prepared from fish oil. Emulsions at lower pH values fell between 0.7 - 1 μm range and were not stable as well while emulsions at higher pH values fell in 0.5 μm range.

3.6.2 PL-90 with sodium oleate in soy oil

Sodium oleate addition had a great effect on the stability of this egg lecithin type with soy oil. Emulsions at higher pH were stable for a period greater than 5 months (at pH 8 and 9) while that of pH 7; formulations were stable around 3 months. PSD curves of emulsions prepared using egg lecithin in soy oil with sodium oleate addition are indicated in Figure 3.28. Particles as low as 250 nm range were measured with this type of lecithin using sodium oleate addition.

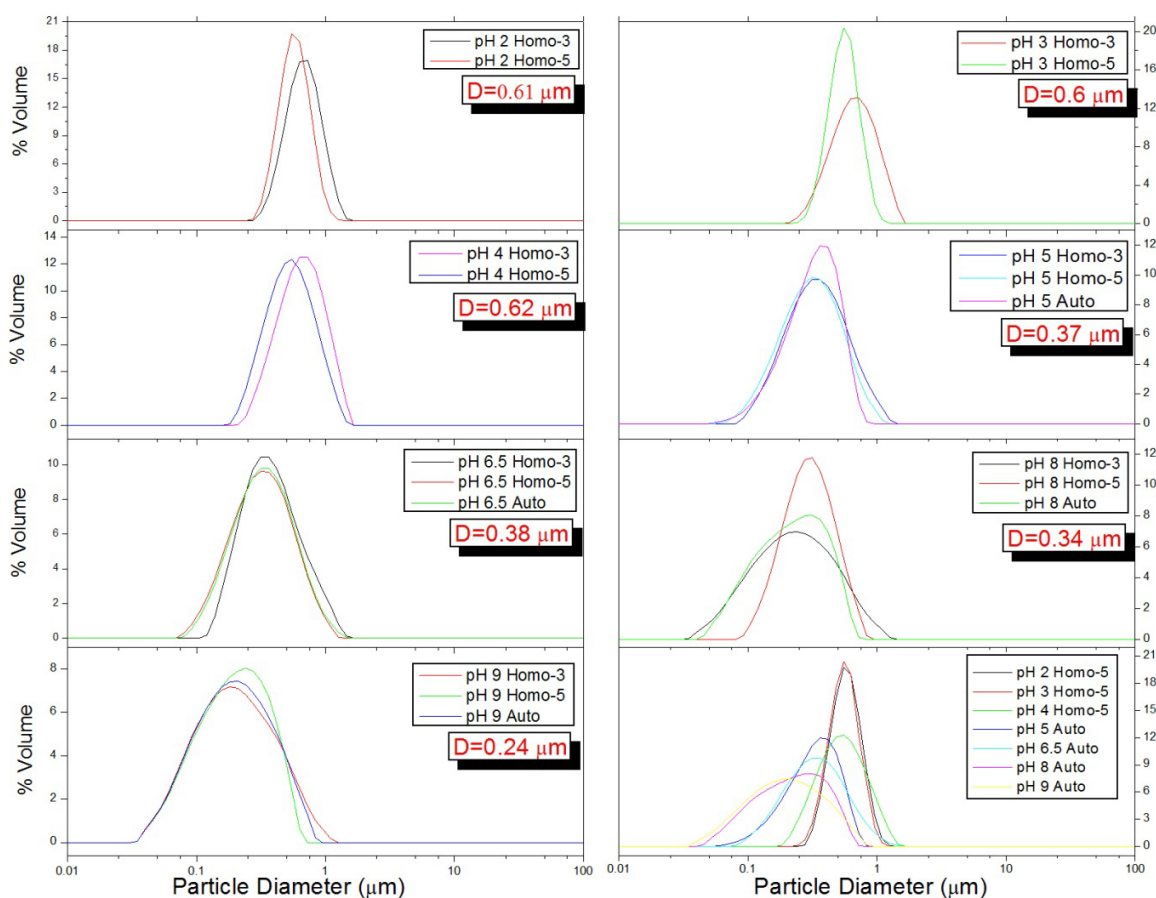


Figure 3.28: Particle size distribution curves for emulsions prepared using Fresenius-Kabi lecithin from Sweden as emulsifier in soy oil with sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

On an informative note, PL-90 is the type of lecithin Fresenius Kabi is using for the production of its famous intravenous (IV) emulsion product commercially known as Intralipid®. Intralipid as indicated in Table 3.1 is stable for more than 2 years with certain additions other than sodium oleate alone (Fresenius Kabi product ingredient guide). A similar stable trend was observed at pH 5 but the results were not repeatable due to the fact that the pH was raised after processing and fell in the stable range of pH 7.

3.6.3 PL-90 in fish oil concentrate

Experiments indicated that the emulsions with fish oil were prone to gel formation at all pH values but were limited to only coarse emulsion phase. Gel structure was distorted as soon as emulsions were homogenized after pre-emulsion stage. This gel structure was pronounced especially at pH 9 and a strong gel cake was observed during coarse emulsion phase.

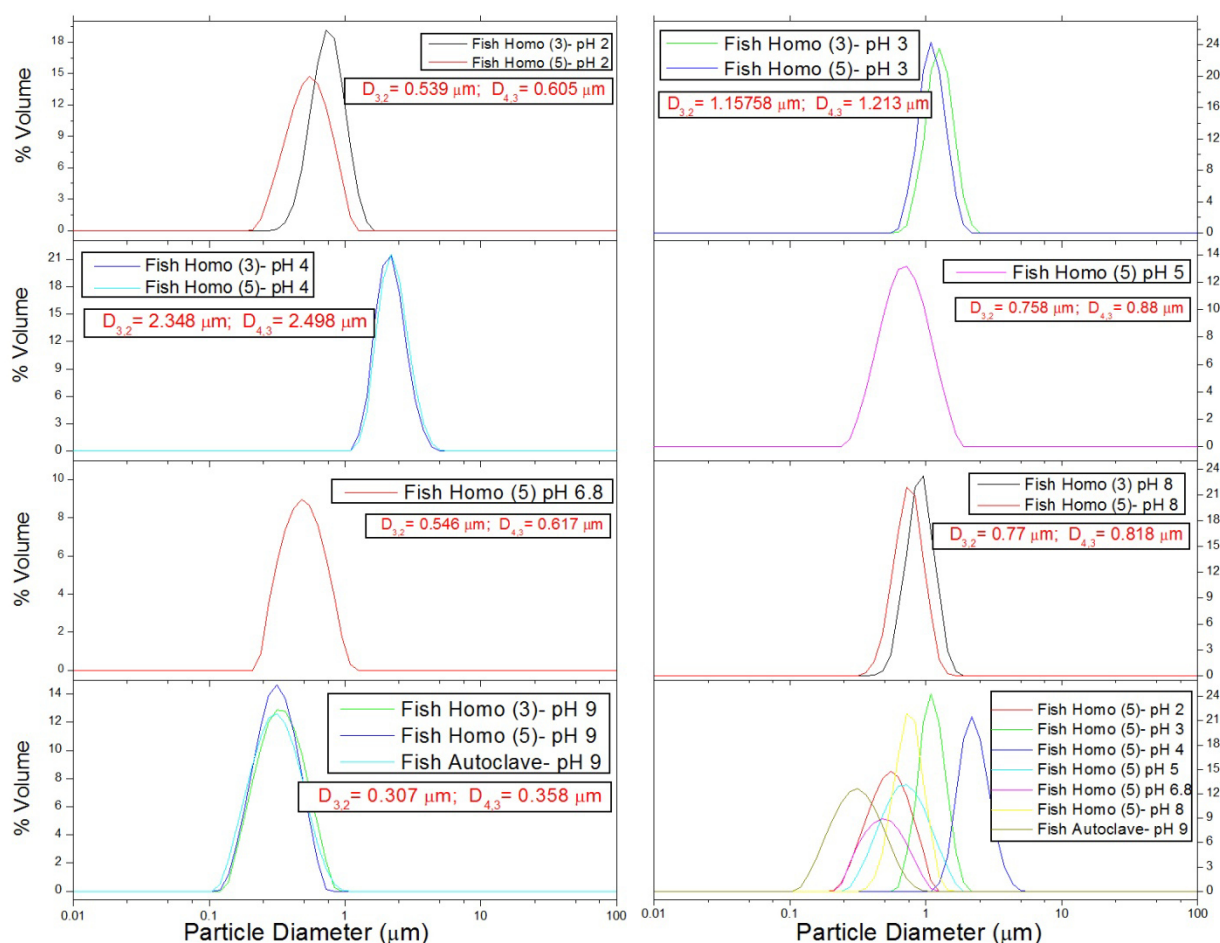


Figure 3.29: Particle size distribution curves for emulsions prepared using lecithin from Fresenius-Kabi Sweden as emulsifier in fish oil as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

Separation mechanism, as observed by visual inspection of emulsions prepared by dissolving egg lecithin in fish oil at pH 9, was a layer of oil at the top with a small layer of water at the bottom while in between is a non-deformable gel. Further processing was only possible by repeating the same emulsion at said pH at faster transition time between coarse and fine emulsion. Care was taken for pH measurement as there may be a chance of measurement error when the pH was measured after coarse emulsion as gel surrounded the electrode which may not indicate the true value of pH in that situation.

Similar to Lipoid E 80 S emulsions in fish oil, there was also a pungent odor of fish oil coming from the samples after homogenization treatment (pH 8 and 9). The color of the emulsions changed to off-white during the passage of time from white and water came out of the samples at bottom at low pH values. A thick cream layer containing oil drops was observed at the top of emulsions at high pH values. No sample survived autoclave treatment while at low pH values, in acidic environment, no sample survived any processing treatment. The particle diameter of emulsions prepared from fish oil using egg lecithin was in the range between 0.5 to 1 μm for low pH and at higher pH, it was measured under 0.5 μm . Figure 3.29 is a brief description of the particle sizes adopted with this lecithin type.

The color anomaly has already been described in section 3.5.6 which was attributed to the oxidation of naked oil drops of fish oil in the formulations reported earlier and hydrolysis of lecithin and its interaction with fish oil oxidation product after diffusing out of emulsifier from the inter-phase.

3.6.4 PL-90 with sodium oleate in fish oil concentrate

Formulations were prepared with only neutral and basic pH values with sodium oleate addition due to the argument made earlier related to the dissolution of sodium oleate at acidic values.

Figure 3.30 shows PSD curves for pH 7, 8 and 9 for fish oil using egg lecithin with sodium oleate addition. Particle size under 200 nm was obtained but the stability of these emulsions was not adequate despite smaller particle size. Smaller particle size was attributed to the lower viscosity of fish oil and also the co-emulsifier properties of sodium oleate as indicated already above.

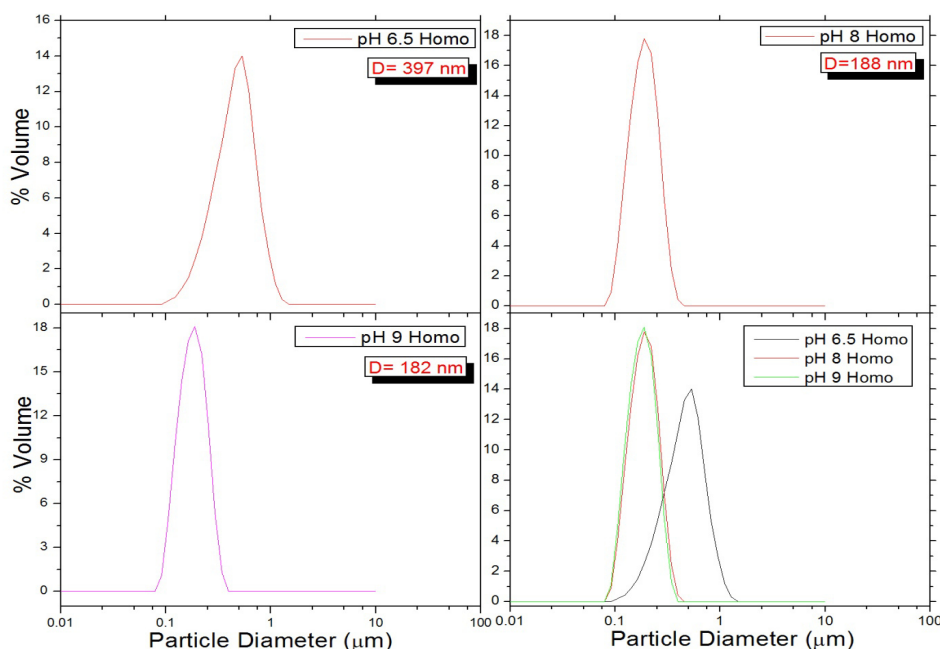


Figure 3.30: Particle size distribution curves for emulsions prepared using Fresenius-Kabi lecithin from Sweden as emulsifier in fish oil with sodium oleate as a function of pH values. (Legend: Homo is homogenization processed samples)

3.6.5 Zeta potential measurements of PL-90 formulations

The information on the composition of this type of egg lecithin (PL-90 from Fresenius Kabi) was kindly supplied by the supplier and Table 2.3 indicates technical data associated to this product. For ζ potential measurements, values were measured with the similar protocol adopted for other type of egg lecithin discussed in section 3.5. IEP was also calculated by fitting a 1st order exponential similar to the ζ - potential curve of Lipoid E 80 S lecithin. It was calculated between 2.8 and 3 pH values. For fish oil emulsions, at pH 2 and 3, emulsions were firstly prepared at neutral environment and then controlling the required pH before homogenization to avoid gel formation and to get ζ - potential values. Figure 3.31 indicates the ζ – potential curves obtained for calculation of IEP for PL-90 lecithin in soy and fish oil respectively.

Same argument of presence of OH^- ions held valid for negative ζ - potential values at higher pH, while on account of information on composition of PL 90, it might be understood that the other causes of negative ζ - potential values were with reference to the composition of lecithin, containing 70% PC. However, change in color of the emulsions with fish oil was a clear indication of either presence of lyso-phosphatidylcholine or hydrolysis of phosphatidylcholine (along aging time). Another cause of change in color and pungent smell might be degradation of fish oil with time as soon as the emulsifying layer was removed from oil-water interface, hence indicating lack of stability, Serfert et al. (2009) and Netz (2004).

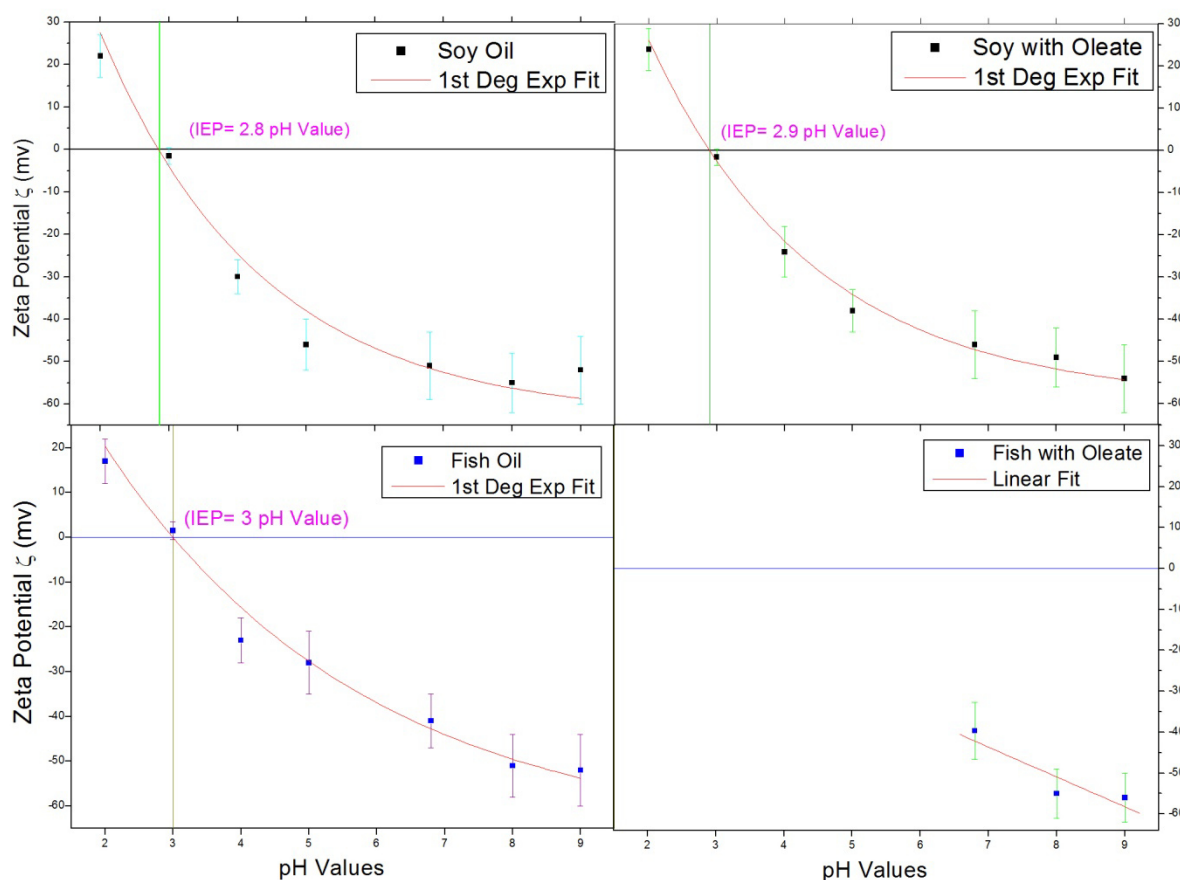


Figure 3.31: Zeta potential values of emulsions from Fresenius-Kabi egg lecithin dissolved in soy and fish oil with and without addition of sodium oleate as a function pH values.

3.6.6 Stability of PL-90 formulations

As indicated earlier in this section, egg lecithin supplied by Fresenius-Kabi gave very poor stability with reference to fish oil. However, soy oil formulations were maximum stable with the help of sodium oleate addition at near neutral and higher pH. Figure 3.32 is a self-indication of stability of emulsions prepared with this egg lecithin.

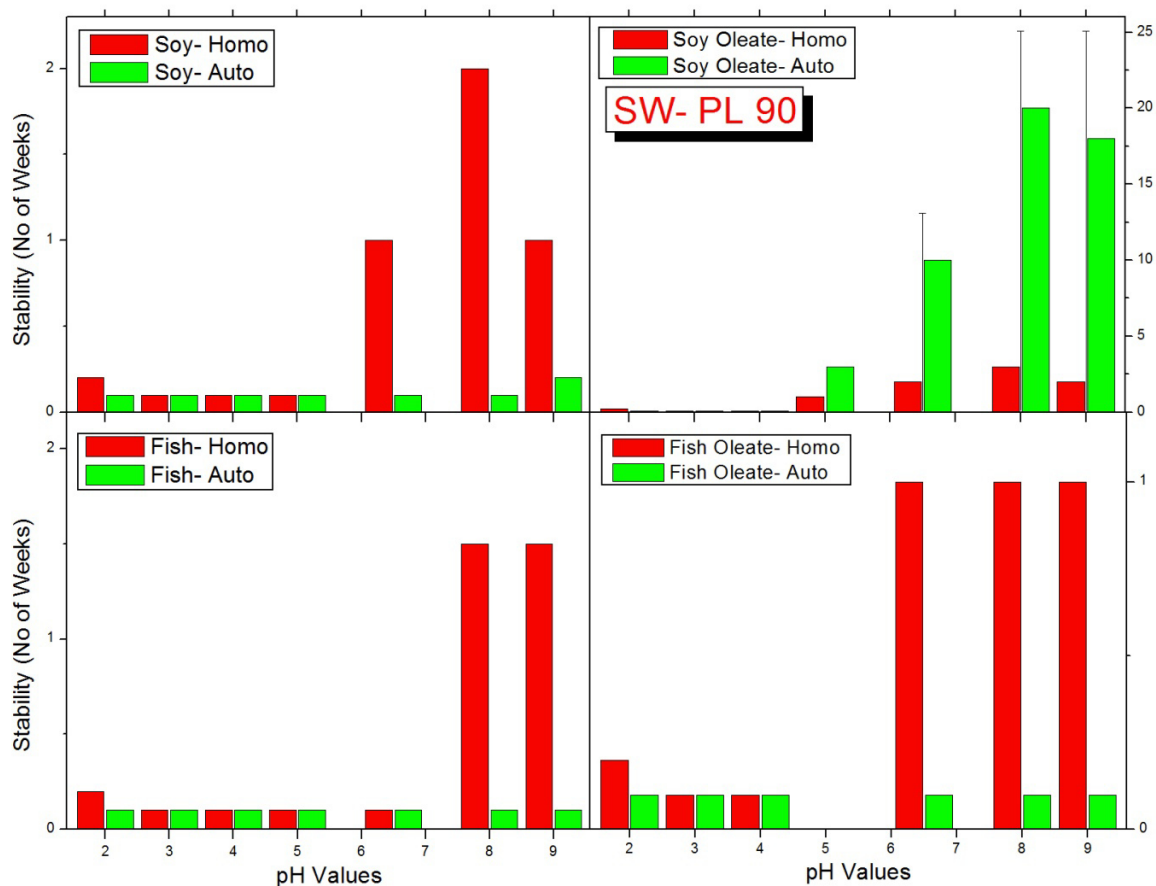


Figure 3.32: Stability of Fresenius-Kabi Sweden egg lecithin emulsion samples dissolved in soy and fish oil with and without co-addition of sodium oleate as a function of pH values.

It has been indicated in section 3.6.2 that fish oil with this type of lecithin gave a strong gel structure at pH 9. The methodology to get emulsions at that pH was changed in order to characterize the emulsions with fish oil dissolved in this lecithin. So, PL.90 was dissolved in water and then emulsion was formed. In any of the case, no stable emulsion was formed, but the gel phase was surely eliminated. Commercially, Fresenius Kabi is dissolving PL-90 in water phase rather than in oil (I&DCF, Pharma div Fresenius Kabi)

Furthermore, in order to check the gel behavior related to lecithin or the oil type (fish oil concentrate) same emulsion at respective pH with natural (refined) fish oil was prepared. In that case, gel phase was not observed and the emulsion was stable for few weeks after autoclave treatment. This observation may conclude that the fish oil concentrate used in emulsion systems with PL-90 was the reason of gel formation at that pH.

Refined/natural fish oil used contained 35% EPA and DHA altogether, while fish oil concentrate contained above 70% EPA and DHA. PSD of 200 nm range was observed by using natural fish oil while ζ - potential was having similar values as that of fish oil concentrate (i.e. -55 ~ -60 mV).

3.7 Rapeseed oil origin Lipoid-R LPC 20 lecithin

Rapeseed origin Lipoid R LPC 20 lecithin contained minimum 20% of phosphatidylcholine (PC) and lyso-phosphatidylcholine (LPC) each, according to the manufacturer (Table 2.4). It was in coarse powdered/granular formation and was not dissolvable in either of the oil types used, fish or soy, even after grinding the granules into fine powder. Hence, it was dissolved firstly in water and then emulsions were made. Also, a brownish-yellow color of emulsions were visually observed at higher pH (8/9) rather than a pure white as compared to other formulations. This color was instantly observed when base (NaOH) was added in the system to control pH of the formulations.

3.7.1 Lipoid-R LPC 20 in Soy oil

A 200-250 nm particle size range was adopted by using R LPC 20 with soy oil. Figure 3.33 is an indicative of particle size obtained from that kind of lecithin.

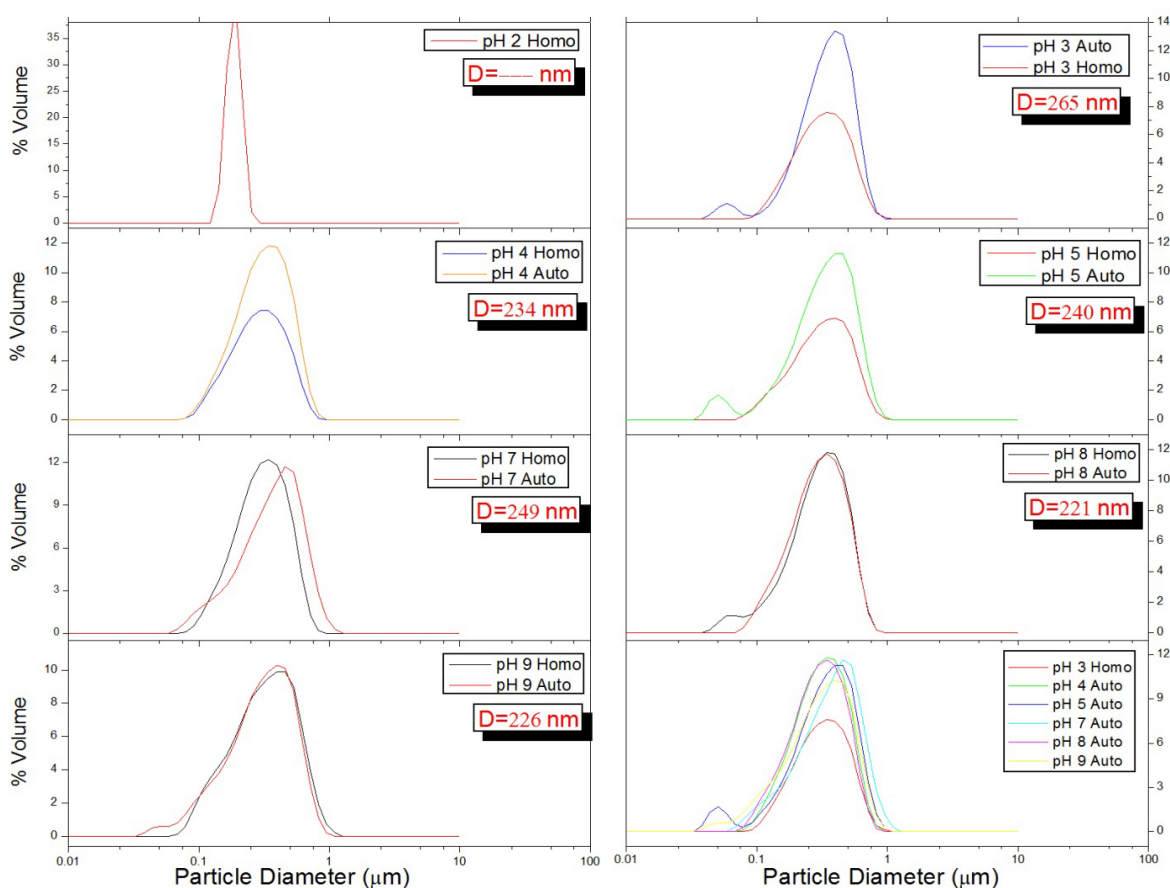


Figure 3.33: Particle size distribution curves for emulsions prepared using Lipoid-R LPC 20 lecithin as emulsifier in soy oil as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

Approximately all of the formulations were stable by using R LPC 20 lecithin in soy oil as indicated in Figure 3.39, at the end of this section, except for pH 2. The measurements made for PSD of pH 2 was out of the sensitivity of the equipment due to extreme poly-dispersivity (separation of phases) and the equipment (Zeta sizer) gave measurement error as indicated in Figure 3.33. The reason of stability at larger pH range may be due to lyso- content of this lecithin (lyso- phosphatidylcholine) as indicated by Kawaguchi et al. (2008) who concluded that lyso-phosphatidylcholine is a better emulsifier than phosphatidylcholine alone.

3.7.2 Lipoid-R LPC 20 with sodium oleate in soy oil

The effect of sodium oleate addition was not much pronounced by its addition in soy oil emulsions using R LPC lecithin as there was no remarkable change in particle size observed indicated in Figure 3.34. Rather, as stated above, lyso-phosphatidylcholine played its part in stabilization of emulsions irrespective of emulsion particle size.

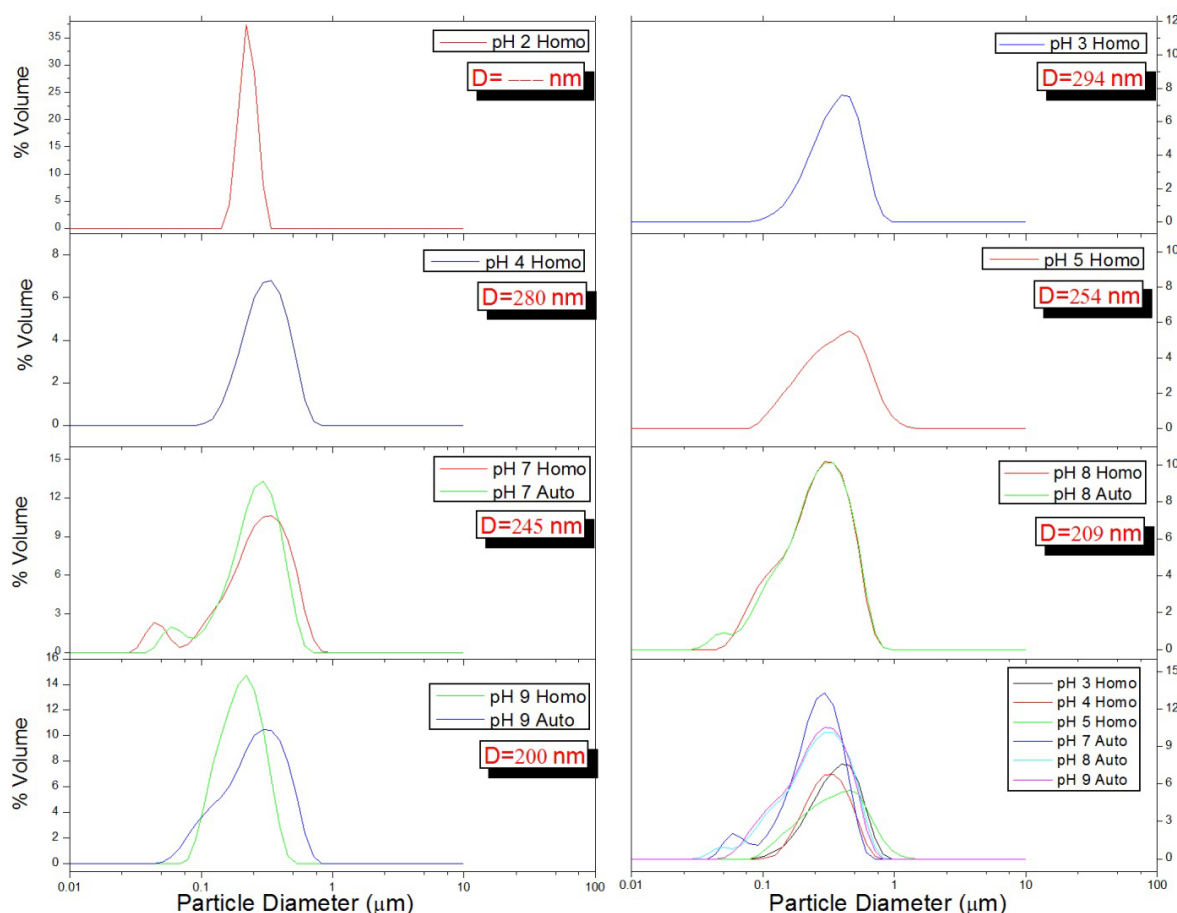


Figure 3.34: Particle size distribution curves for emulsions prepared using Lipoid-R LPC 20 lecithin as emulsifier in soy oil with co-added sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

Figure 3.39 indicated that the stability was compromised when sodium oleate was used at lower pH values and emulsions were no more stable at acidic values, those originally were stable without its addition. The comments have already been made with reference to non- effectiveness of sodium oleate addition at lower pH in section 3.3 and 3.4 (Werling et al. (2008).

3.7.3 Lipoid-R LPC 20 in fish oil concentrate

Due to the presence of lyso- phosphatidylcholine and also the low viscosity of fish oil, the particle size obtained with emulsion using R LPC 20 gave a very narrow particle size lower than a 200 nm mark. The stability of fish oil emulsions was basically attributed to not only smaller particle size but also presence of large amount of LPC. Similar to soy oil emulsions, fish oil emulsions were also stable to a wider range of pH values used in current experimentation, indicated in Figure 3.38 in section 3.7.6.

Figure 3.35 indicates below PSD curves of the formulations prepared with R LPC 20 lecithin with fish oil concentrate. PSD of pH 3, not mentioned in the graph below, was calculated as 213 nm.

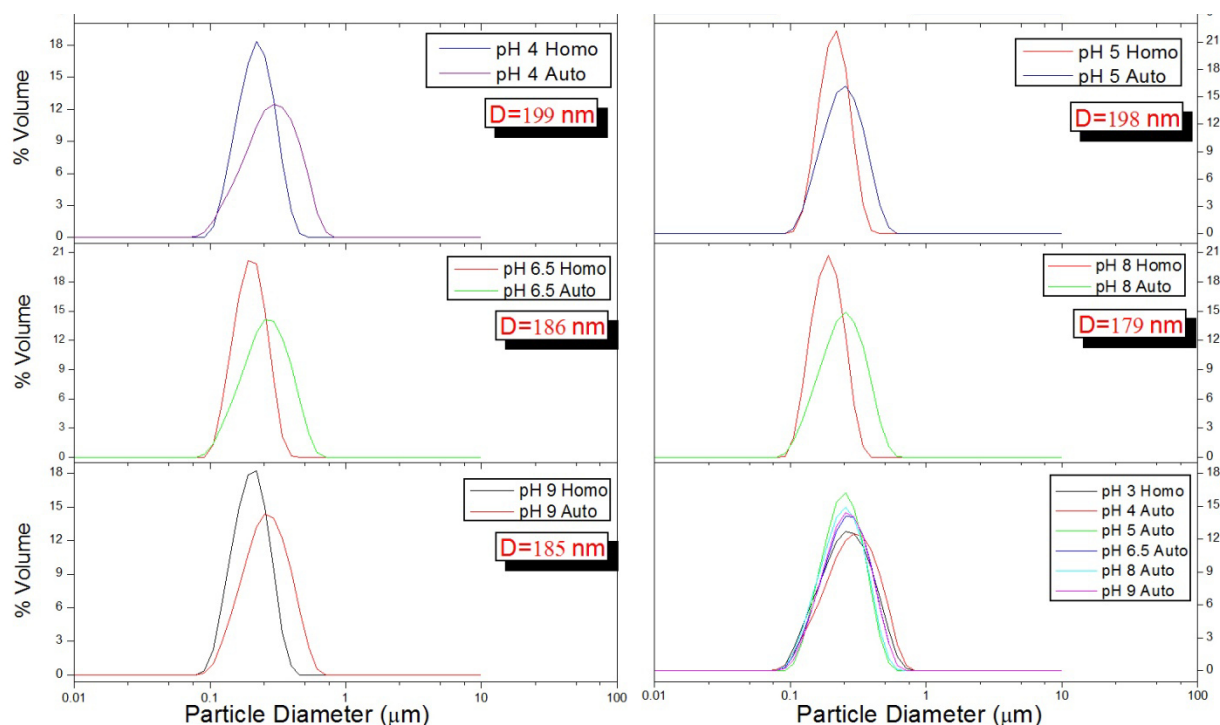


Figure 3.35: Particle size distribution curves for emulsions prepared using Lipoid-R LPC 20 lecithin as emulsifier in fish oil as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.7.4 Lipoid-R LPC 20 with sodium oleate in fish oil concentrate

Emulsion particle size with sodium oleate addition was in the same range as that of emulsions without its addition. Again stability was compromised at near neutral and lower pH values which were made by the addition of sodium oleate. Sodium oleate presence in the solution at lower pH values have been discussed already above in this dissertation. By the addition of sodium oleate with R LPC 20 fish oil emulsions, stable emulsions were not produced as compared to the same formulations without its addition. This might be discussed in more detail at the end of this Section (3.7.6). Figure 3.36 shows PSD curves obtained for emulsions using R LPC 20 lecithin in fish oil with sodium oleate. PSD at pH 5, not presented in the graph below, was calculated as 193 nm.

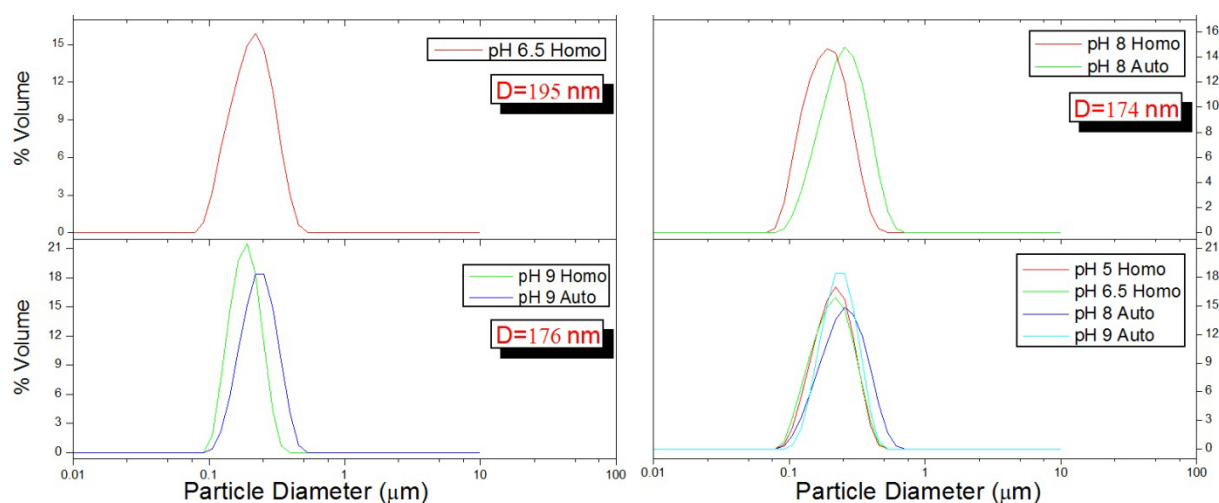


Figure 3.36: Particle size distribution curves for emulsions prepared using Lipoid-R LPC 20 lecithin as emulsifier in fish oil with sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

As indicated earlier, sodium oleate addition gave smallest PSD at higher pH values; an evolution of PSD along time for pH 9 with sodium oleate added fish oil emulsion is also presented here in Figure 3.37. From the time of preparation till it becomes poly-disperse (in this case, after 5 days) while transition from being poly-disperse and getting out of the range of Zeta-sizer and in the meantime measured with Master sizer the next day.

The argument of using Zeta sizer and Master sizer is already explained in Section 3.3.4. It is to be noted that the autoclave treated sample did not change its particle size even after passing 3 weeks as compared to the particle size measured initially after 24 hrs. Similarly, R LPC 20 homogenized emulsion at pH 9 was the only emulsion whose particle size, although poly-disperse, but did not change from 1 week till 3 weeks as shown in Figure 3.37. Other emulsion samples (S-45, S-75 and H-50) changed their particle size along the course of time as shown in their respective Sections (3.3, 3.4 and 3.8).

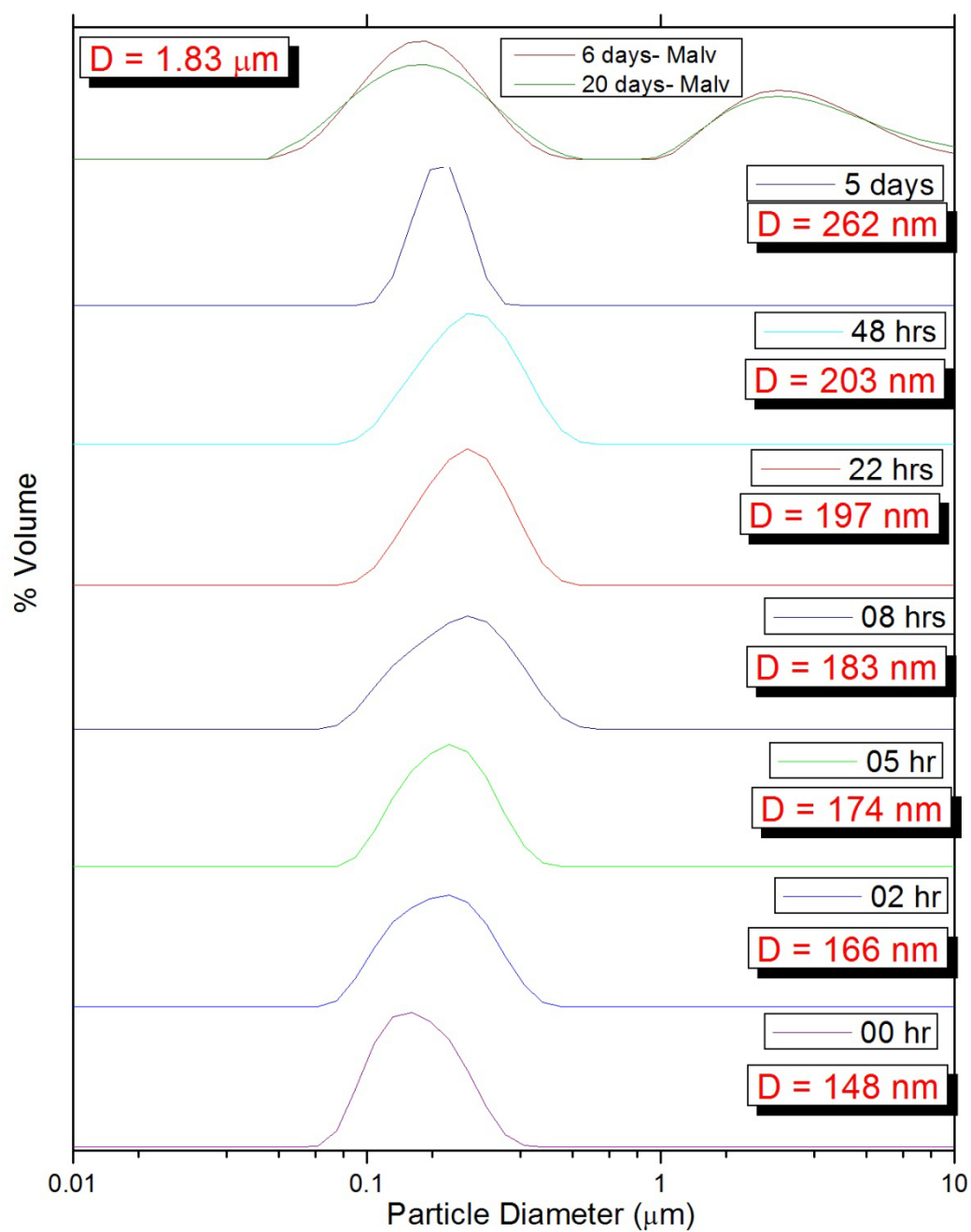


Figure 3.37: Evolution of PSD curves for H 50 lecithin with sodium oleate addition with fish oil at pH 9 with respect to time.

3.7.5 Zeta potential measurements of Lipoid-R LPC 20 formulations

DLVO theory held valid in the formulations obtained by R LPC lecithin with reference to ζ -potential and stability. An exception might be with the highly negative values of fish oil at lower pH by the use of sodium oleate in the systems that were not stable. A great deal of absolute negative ζ -potential values indicated stability to the systems. Plateau region was found for all formulations which was between 4 to 8 pH values for soy oil emulsions with and without sodium oleate addition and between 5 to 8 pH values for fish oil with sodium oleate addition.

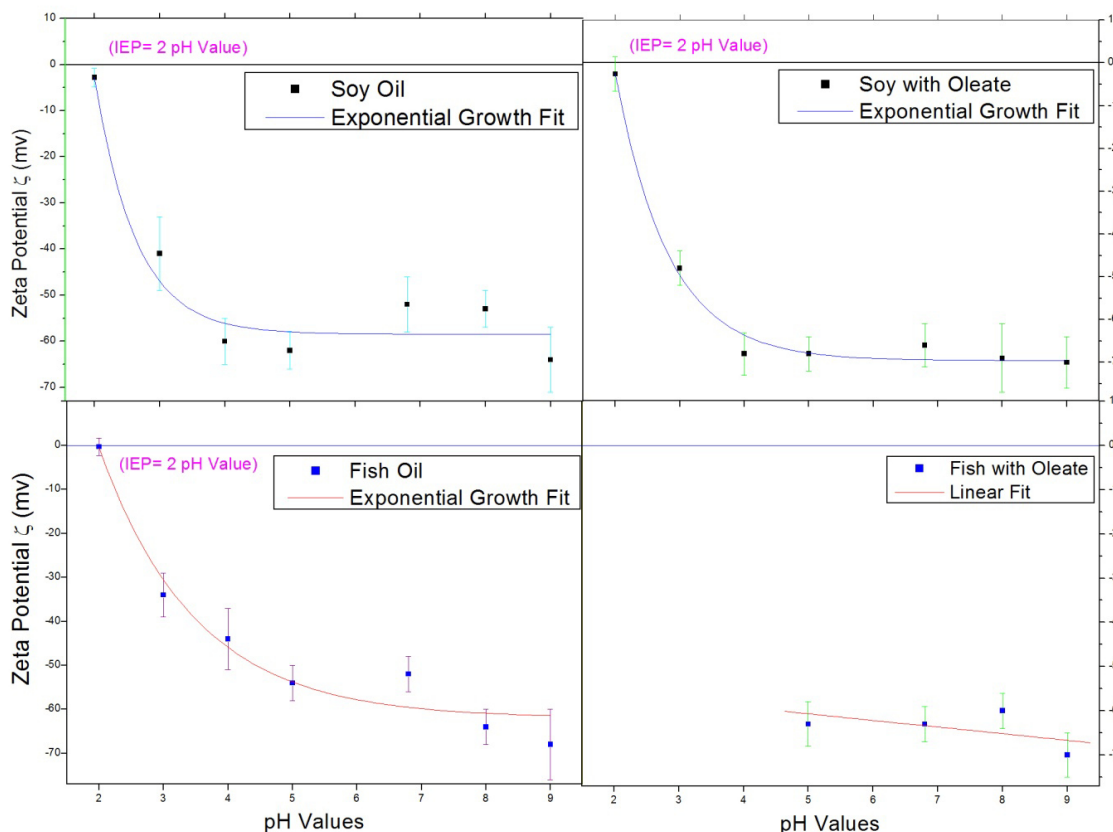


Figure 3.38: Zeta potential values of rapeseed origin Lipoid-R LPC 20 lecithin emulsion samples dissolved in soy/fish oil with and without co-addition of sodium oleate as a function of pH values.

The higher absolute ζ -potential values were attributed to the OH^- ions, oleic ions for sodium oleate added emulsions and due to the charge on LPC at higher pH values, while similar higher ζ -values at lower pH might be attributed to the impurities present in the lecithin. Alonso (1997) and Mosqueira et al. (2000) made phosphatidic acid (PA) responsible for this behavior. According to the supplier, information for only PC and LPC contents were available for this lecithin. Heurtault et al. (2003) indicated in their work that presence of 2-3 % PA might be responsible for higher ζ -potential values.

The use of buffer in case of R LPC was also abandoned due to the fact that the lecithin itself, when added to water that lowered the overall pH of the solution (from neutral to 5 pH value). In this way, adding sodium oleate at higher pH values also demanded tighter control on pH of emulsions, as the two (sodium oleate and R LPC) had opposite behavior in the solution together.

Figure 3.38 shows ζ - potential measurements obtained from emulsions prepared in R LPC lecithin. The calculation of IEP was a bit easier as the charge reversal point was itself measured during measurement at pH 2 for soy/fish oil while also for soy with sodium oleate formulations.

3.7.6 Stability of Lipoid-R LPC 20 formulations

A brief stability picture for emulsions using R LPC lecithin at different pH values with fish and soy oil, with or without addition of sodium oleate can be seen in Figure 3.39. As indicated in section 3.7.4, sodium oleate was not helpful in further stabilizing these formulations due to the presence of LPC in the lecithin composition served the purpose appropriately. It seemed that the co-emulsifier/stabilizing properties of sodium oleate were compromised when it was added with R LPC lecithin and gave lower stability as compared to its effectiveness using other types of lecithin.

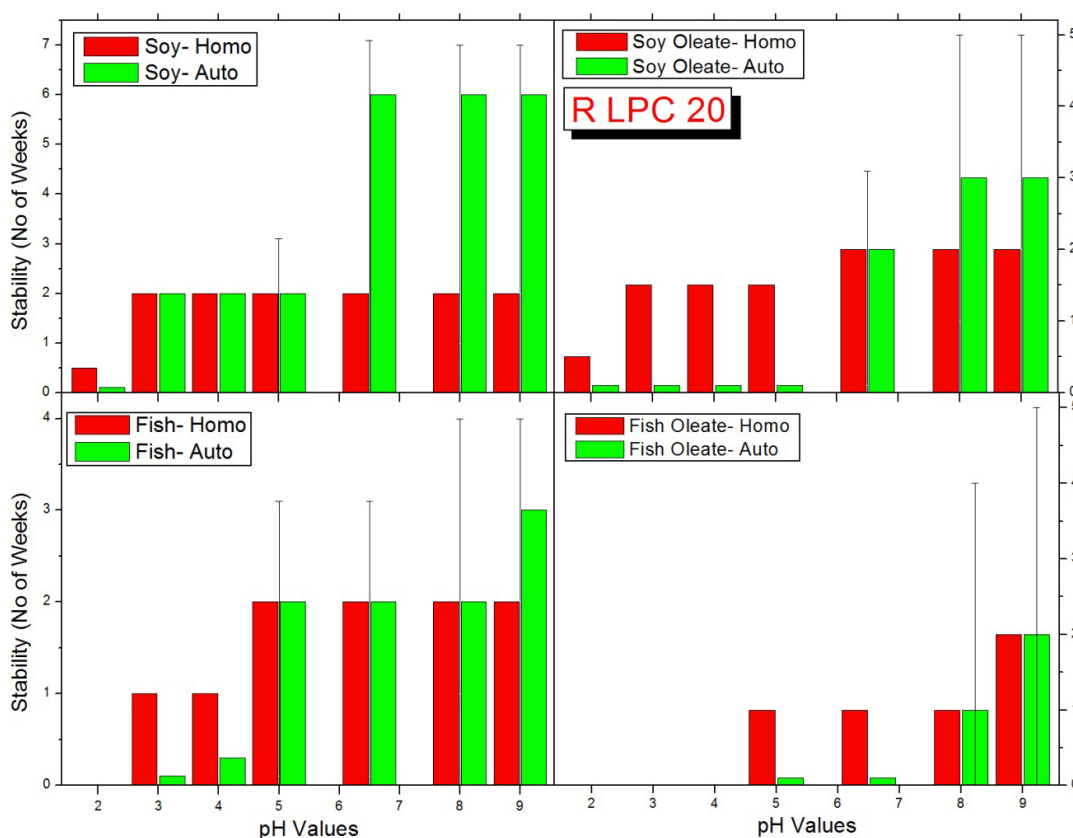


Figure 3.39: Stability of Lipoid-R LPC 20 rapeseed lecithin emulsion samples dissolved in soy and fish oil with and without co-addition of sodium oleate as a function of pH values

There was a clear difference in the emulsions using R LPC lecithin and other types of lecithin with reference to the LPC content and hence change in ζ - potential values, stability and characteristics. This might be attributed to the fact that other types of lecithin acquired LPC by the hydrolysis of PC in the solution and hence the stability of these emulsions might be compromised along time due to the influence of hydrolysis on the emulsions. On the other hand, R LPC 20 itself has 20% LPC in its constituents affecting ζ - potential and stability during aging.

Kawaguchi et al. (2008) confirmed that LPC has greater emulsifying properties. Particularly in reference to parenteral intravenous (IV) fat emulsions, the observations and conclusions drawn by Reman and Vandeene.LI (1967) and Matsuzaki et al. (1988) were of extreme importance who concluded that LPC, due to its greater emulsifying properties and high solubility, causes hemolysis and hence, any fat emulsion using LPC as emulsifier cannot be used as parenteral emulsion.

3.8 Sunflower origin Lipoid H-50 lecithin

Sunflower lecithin contained minimum 50% phosphatidylcholine and 5% phosphatidylethanolamine as indicated in Table 2.4. It has small brown lumps and gave a yellowish brown color at high pH values when titrated with NaOH to maintain/control pH during processing. This characteristic was unique for R LPC and H 50 types of lecithin. H 50 was dissolved in oil and it also lowered the overall pH of the system when added to emulsions as was the case with R LPC type. Emulsions were made with natural fish oil as well for comparison purposes and with buffer solution too, in order to discuss the effect of buffer solution to ζ - potential values.

3.8.1 Lipoid H-50 in Soy oil

A wide range of pH formulations were stable using H 50 lecithin in soy oil and a particle size of 200-300 nm range was observed for the emulsions prepared. Emulsions at pH 2 and 3 were not stable at all as indicated by their poly-disperse particle size range (Figure 3.40). Also, formulations showed a gel behavior at pH 2. Near neutral and basic pH values gave maximum stability in these formulations, indicated in Figure 3.46 at the end (section 3.8.6).

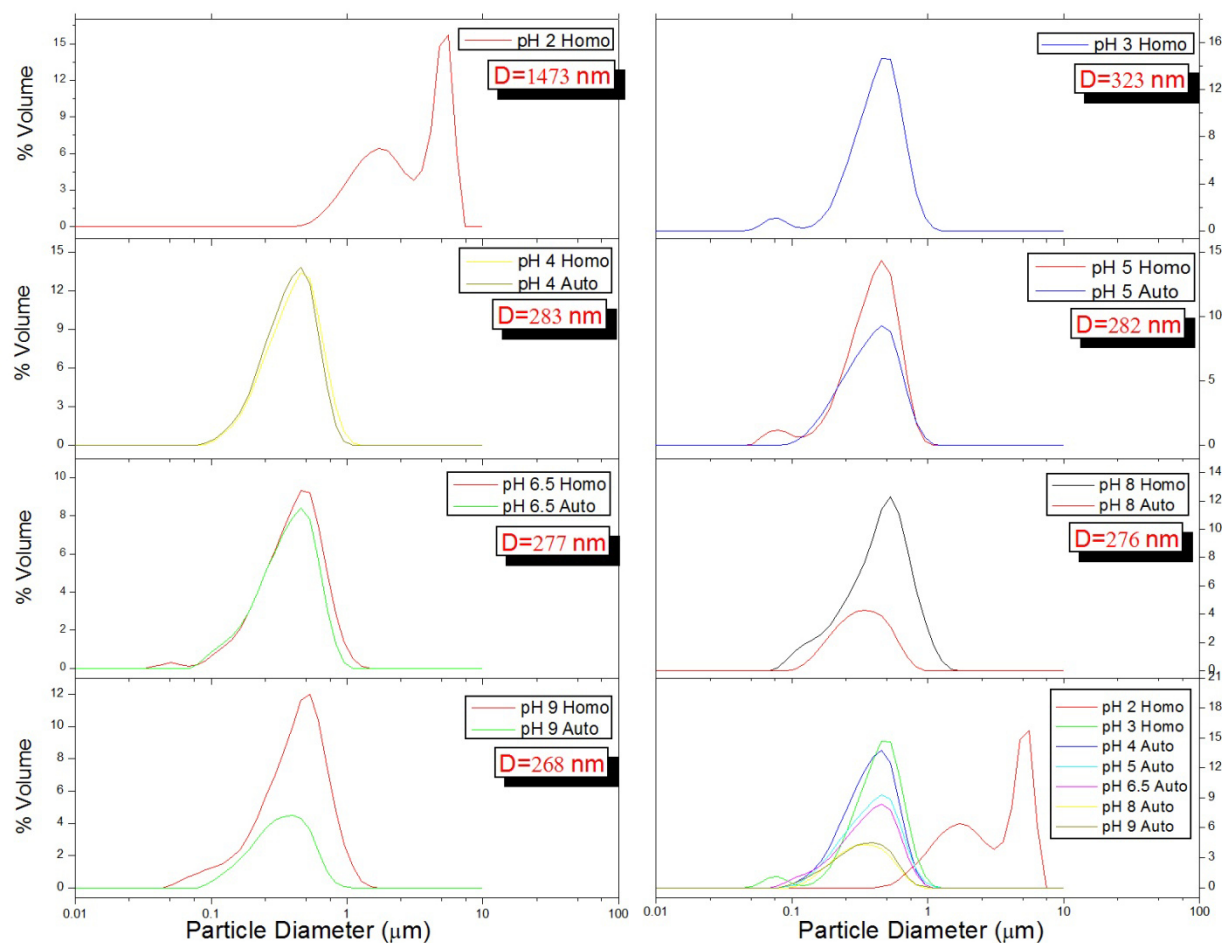


Figure 3.40: Particle size distribution curves for emulsions prepared using Lipoid H-50 lecithin as emulsifier in soy oil as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.8.2 Lipoid H-50 with sodium oleate in soy oil

Although particle size distribution was improved by using sodium oleate in soy oil emulsions with H 50 lecithin, stability was observed lower as compared to the emulsions prepared without its addition. This type of lecithin contains 50 % of phosphatidylcholine in its composition as indicated in Table 2.4, with marginal phosphatidylethanolamine content. A 200 nm particle size was observed as indicated in the distribution curves in Figure 3.41. Emulsions prepared at acidic pH values did not survive the autoclave treatment, for the fact that sodium oleate was not in the solution at lower pH values due to compromise on its solubility as discussed earlier. Stability graph has been presented at the end of this section in Figure 3.46. Also, particle size at pH 2 was out of the sensitivity of the zeta-sizer due to its poly-dispersivity.

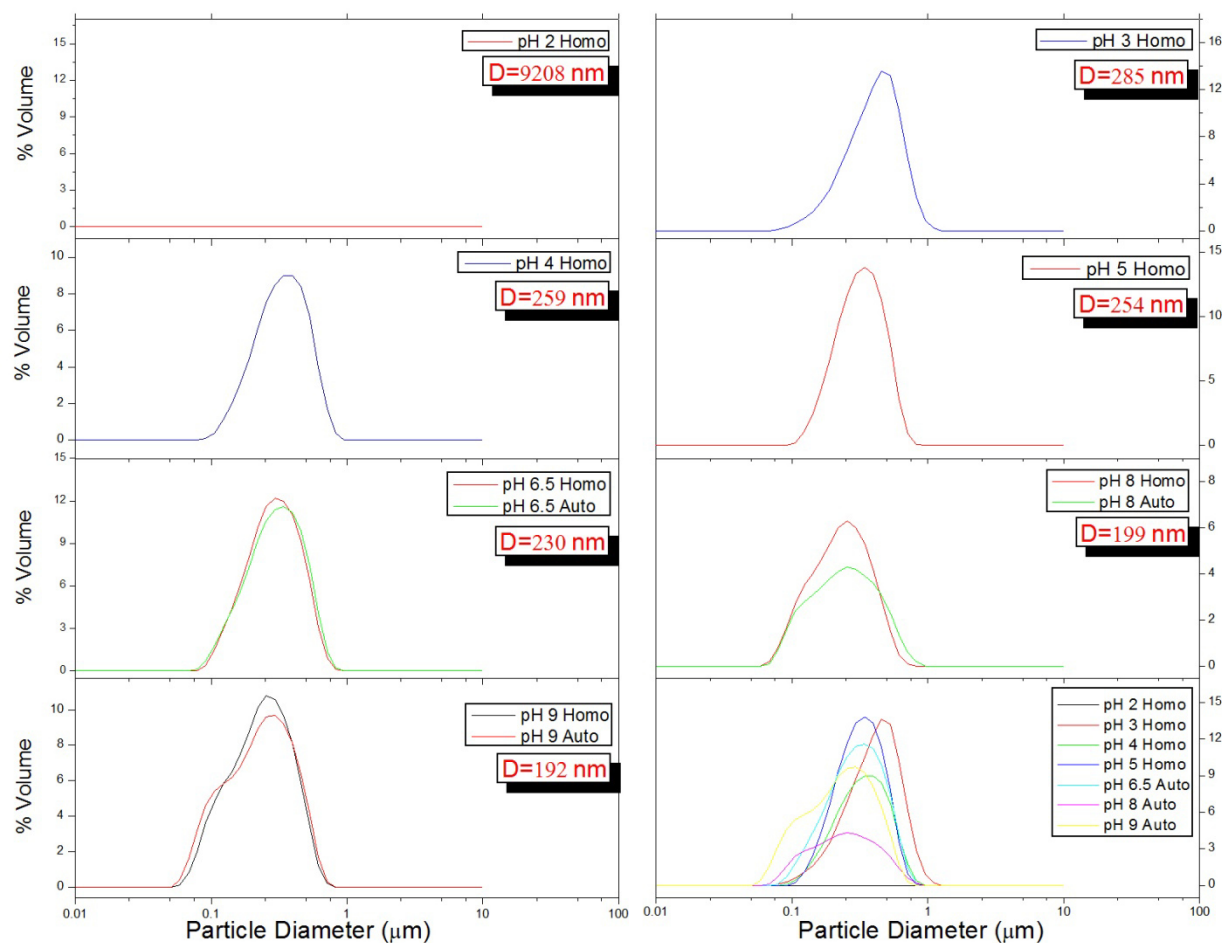


Figure 3.41: Particle size distribution curves for emulsions prepared using Lipoid H-50 lecithin as emulsifier in soy oil with co-added sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.8.3 Lipoid H-50 in fish oil concentrate

H 50 formulations were also stable at higher and near neutral pH values when fish oil was used. A PSD of as low as 180 nm was indicated at pH 9 using fish oil emulsions. It has already been discussed that low viscosity of fish oil helped decreased particle size in most of pH environments as indicated by the PSD curves of H 50 formulations using fish oil in Figure 3.42 below. The stable life was around 2-3 weeks for the fish oil emulsions (see Figure 3.46).

Due to gel behavior of H 50 formulations at lower pH values, emulsions were prepared at pH 2 by using only natural fish oil (containing 35% max EPA and DHA). Larger particle size was obtained in the range of 1 μm when this oil type was used which was attributed to its difference in viscosity with not only water but also with the fish oil concentrate used initially (Solutex 0765). There was no gel observed at that pH value (2 units) and the homogenized emulsion

samples survived for a week time. ζ - Potential values were in the same range as were with 0765 fish oil concentrate i.e. +7 mV ~ +10 mV at pH 2 with natural fish oil.

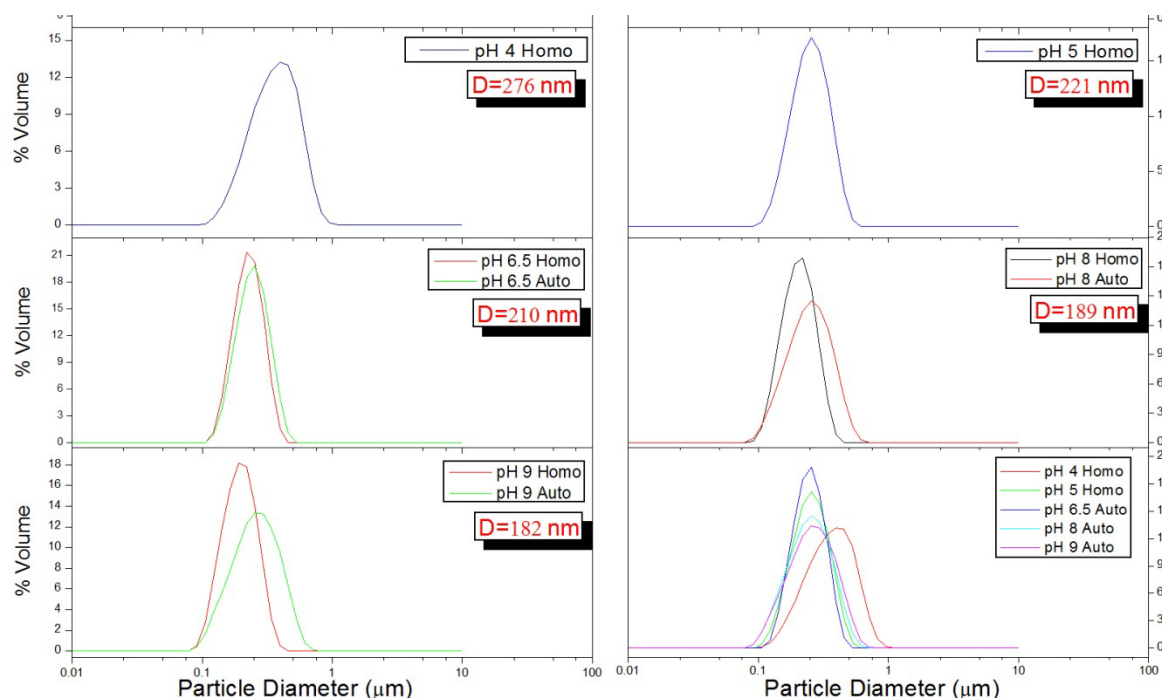


Figure 3.42: Particle size distribution curves for emulsions prepared using Lipoid H-50 lecithin as emulsifier in fish oil as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

3.8.4 Lipoid H-50 with sodium oleate in fish oil concentrate

When sodium oleate was added with the fish oil emulsions prepared with H 50 lecithin, likewise all other lecithin types, there was a pronounced decreased in particle size observed specifically at higher pH values. Figure 3.43 indicates the smallest particle size distribution observed using sodium oleate in fish oil emulsions and a distribution as low as 150 nm was obtained. Stability of these emulsions with sodium oleate was compared to the formulations without its addition and 2-3 weeks life span was adopted at higher/near neutral pH. Smaller particle size was also prone to agglomeration and flocculation and a transition of this nano-metric particle size to poly-disperse distribution is indicated in Figure 3.44 at pH 9. PSD at pH 3, not mentioned in Figure 3.44 was calculated to be 343 nm.

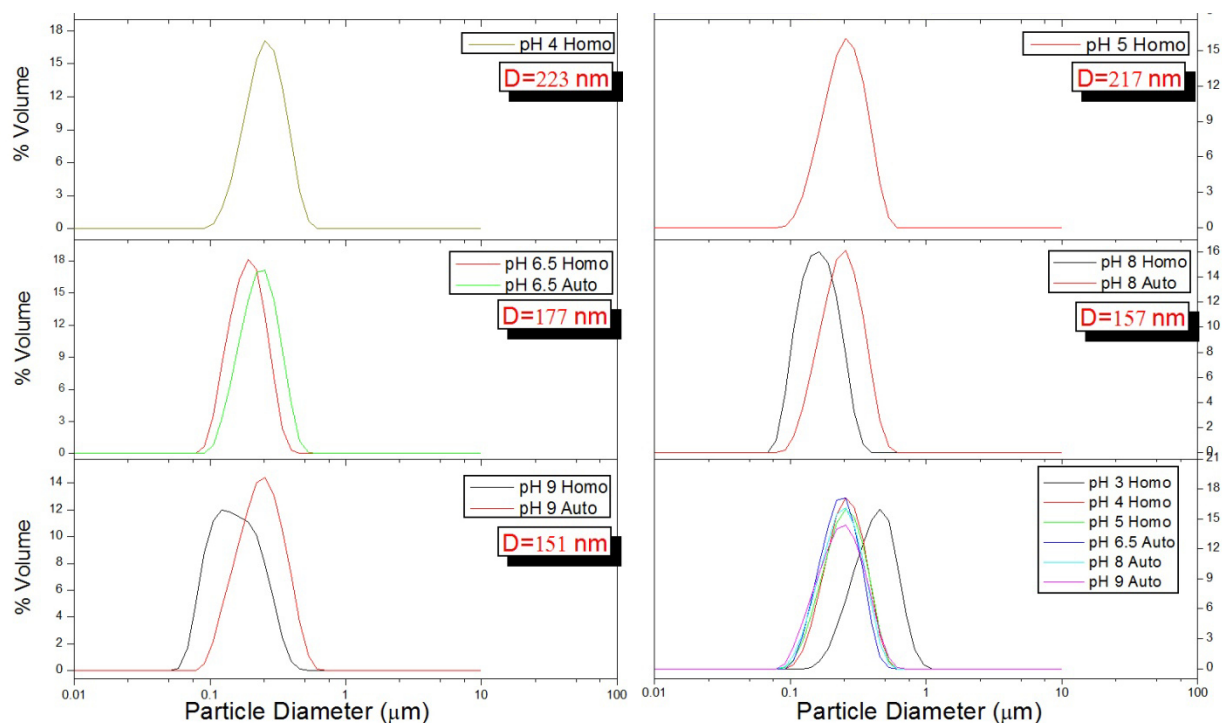


Figure 3.43: Particle size distribution curves for emulsions prepared using Lipoid H-50 lecithin as emulsifier in fish oil with co-added sodium oleate as a function of pH values. (Legend: Homo is homogenized sample while Auto is autoclave treated samples).

After 5 days, the particle size measured was out of the range of Zeta-sizer due to the polydispersity and Master sizer was used to determine it (Figure 44). It should be noted that Master sizer measurement of PSD under 200 nm was out of the sensitive range of this equipment and it measured no significant change in particle size along time till day 5/6 but when the PSD fell in effective range of this equipment, more than 200 nm, it observed changes, as indicated already in Section 3.3.4.

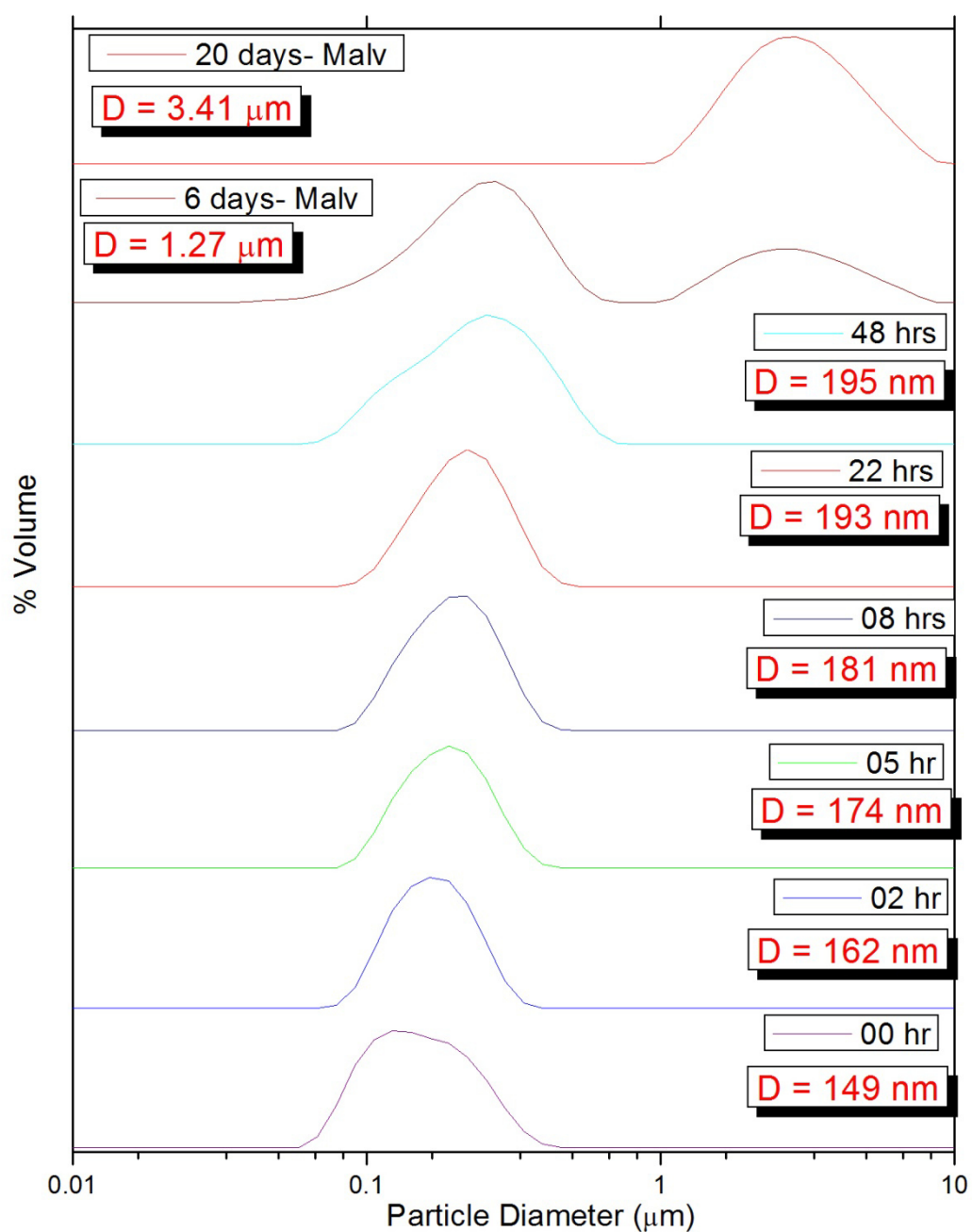


Figure 3.44: Evolution of PSD curves for H 50 lecithin with sodium oleate addition with fish oil at pH 9 with respect to time.

3.8.5 Zeta potential measurements of Lipoid H-50 formulations

Transformation of charge from negative to positive ζ - potential values at pH units between 2 and 3 for the formulations prepared with H 50 using soy and fish oil with and without addition of sodium oleate. DLVO theory held valid in the formulations obtained with reference to ζ - potential and stability. Negative values of fish oil emulsions with sodium oleate may be an exception at lower pH that was not stable. The role of sodium oleate at acidic pH has already been explained by Werling et al. (2008) earlier. A great deal of negative ζ - potential values indicated stability to the systems. At pH 7, the ζ - potential value was out of phase due to near neutral atmosphere and a lack of control of the pH of formulation due to H-50 lecithin characteristics (see Section 3.7 with reference to decrease in pH by addition of lecithin i.e. R LPC 20). This fact was only pronounced at higher and near neutral pH values. At negative/acidic pH values, there was no change due to already at acidic environment.

As discussed earlier, the higher ζ - potential values at basic pH were attributed to the OH^- ions, anionic oleic ions for sodium oleate added emulsions and might be due to the charge on impurities present in the lecithin. Alonso (1997) and Mosqueira et al. (2000) made phosphatidic acid (PA) responsible for this behavior. According to the supplier, there was only info related to the PC (50%) and PE (5%) content of this lecithin in question while the entire composition was not available. However, Heurtault et al. (2003) indicated in their work that presence of 2-3 % PA might be responsible for higher ζ - potential values.

Figure 3.45 is a graphical presentation of ζ - potential measurement obtained from emulsions prepared with H 50 lecithin. Charge reversal point, as indicated above, was between pH 2 and 3 for soy and soy with sodium oleate formulations. Natural fish oil emulsions, at pH 2 gave positive ζ - potential values, as indicated in section 3.7.3.

Buffer solution was also used to prepare the formulations using H 50 lecithin at two different pH values i.e. pH 5 and pH 8. It must be noted that at lower (acidic) pH values, the use of buffer did not affect the ζ - potential values due to the fact that the presence of ions at lower pH were already too high by the addition of large quantity of 1N HCl to control pH. The addition of salt to prepare a buffer gave no difference in the ζ - potential values. This effect was experimentally observed during measurement of ζ - potential at lower pH values by observing the conductivity of the sample emulsion in question.

The conductivity was also measured during ζ - potential measurement with magnitude between 0.01 - 0.1 mS/cm, for pH values from 4 – 9. As soon as the salt was added to make buffer solution at any of the pH value (from 4 - 9), the conductivity jumps to more than 3-4 mS/cm. But for pH 2, addition or non-addition of salt did not influence the conductivity and the range remained 3-4 mS/cm. Meanwhile, the ζ - potential values obtained were far different from the emulsion samples using buffer solution as compared to the ones without using buffer solution and in-fact, the values were decreased when buffer was used.

An exception to this behavior was sodium oleate addition in the formulations. It has been already discussed earlier that sodium oleate, when used, raised the overall pH of the systems and hence acted as a buffer. Werling et al. (2008) emphasized the need to further research the buffer behavior of sodium oleate. But it should be noted that there was no effect on the conductivity of emulsions when sodium oleate was added and ζ -potential measurements were carried out. The effect of electrolytes addition on ζ -potential values has already been discussed earlier and practical evidence is presented here for emulsions using H 50 lecithin.

Two different buffers have been used to prepare emulsions at pH 8 (with sodium salt and with potassium salt). The answer to the question why using two different buffer was due to the obvious fact that the amount of electrolytes present in a solution are affected by the type of salt addition, and hence the quantity of it too, to prepare the buffer, hence affecting ζ -potential due to electrolyte quantities. Unfortunately, the only soy emulsions survived was with buffer using potassium salt when H 50 was used at pH 8 and it was not possible to demonstrate the effect of buffer solution on ζ -potential values. It should be noted that potassium salt quantity was half of the sodium salt used to prepare the same buffer, affecting ζ -potential and stability too. Formulations were prepared with pH 5 using acetate buffer but they did not survive, similar to the S 45 and S 75 emulsions.

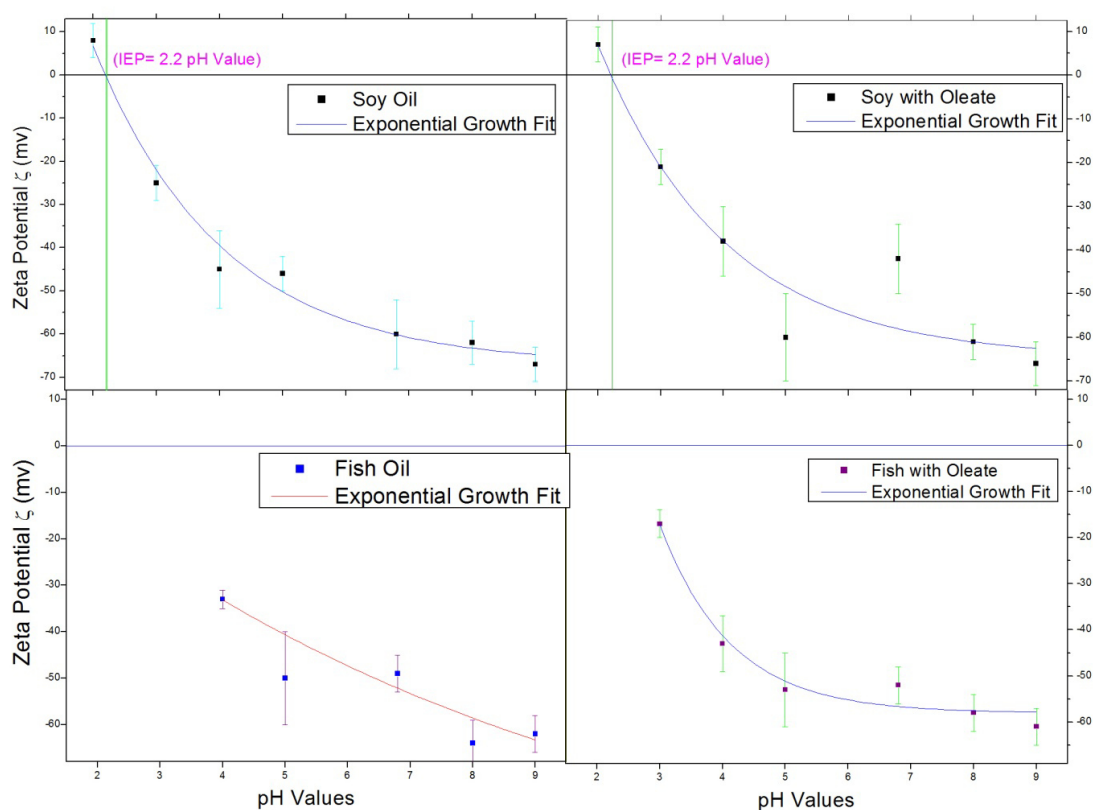


Figure 3.45: Zeta potential values of sunflower origin Lipoid H-50 lecithin emulsion samples dissolved in soy and fish oil with and without co-addition of sodium oleate as a function of pH values).

A conductivity of 9-14 mS/cm was observed with Na/K buffer during ζ - potential measurement as compared to only 0.01 mS/cm without buffer solution and a value of ζ - potential as low as -26 mV and -17 mV for Potassium and Sodium salt respectively, compared to -60 mV without a buffer. Decrease in ζ - potential values by using buffer has been reported by various authors indicated above and were in-line with the experimental observations during experimental work in the current research (Freitas and Muller (1999); Aoki et al. (2005) and Muller and Heinemann (1992).

3.8.6 Stability of Lipoid H-50 formulations

A graphical presentation for emulsions using H 50 lecithin at different pH values with fish and soy oil with and without addition of sodium oleate is indicated in Figure 3.46. As indicated in section 3.7.4. Sodium oleate affect was not significant to further stabilize these formulations as the life span was approximately similar. However, a reduced particle size was observed though.

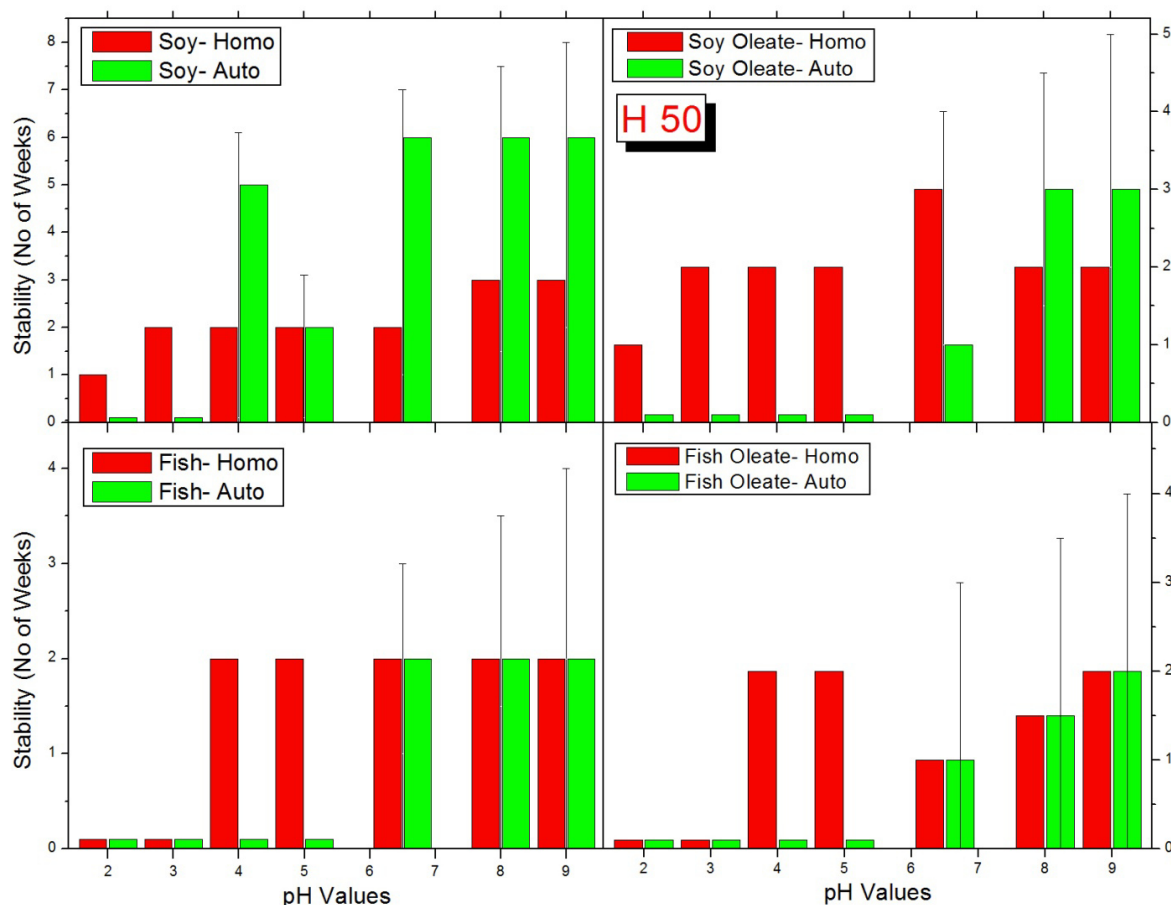


Figure 3.46: Stability of Lipoid H-50 sunflower lecithin emulsion samples dissolved in soy and fish oil with and without co-addition of sodium oleate as a function of pH values.

4. Conclusion

The experimental data for emulsions prepared at a range of pH by using different origin lecithin have been presented in this section with fish and soy oil. The results have been compiled with reference to the PSD and ζ - potential measurements for these emulsions. The stability of these emulsions was hence discussed along with the range of pH in relation to PSD and ζ - potential following creaming (instability) protocol of McClements (1999). Keeping in mind the above experimental results, following conclusions can be drawn:

- Range of pH studied was to establish the basic know how of the lecithin emulsion systems other than the commercial emulsifiers existing in the market. The point of charge reversal, IEP, was a milestone achieved in the current investigation.
- Compared to the commercial products in the market related to IV formulations, the lecithin systems studied with simple three components did not give a comparable stability results but it was very interesting to note that the formulations produced similar characterization results as of commercial products. However, it was very obvious that amongst the range of pH studied in current study, basic pH emulsions have the most stability. Basic pH systems are in terms IV emulsions and have the same pH values considered in the commercial products.
- Stability of Intralipid® and the stability of the same system studied with the commercial egg lecithin (PL-90) did not have the same shelf life due to the following facts in comparison:
 - In three components systems, no anti-oxidant, tocopherol and most of all no hydrolysis suppressant have been used, hence the un-appropriate results.
 - Hydrolysis though helpful in increase in ζ – potential of systems, however, hydrolysis always indicates instability of systems due to undesired reactions
 - Finally, Inert packaging with nitrogen and/or inert atmosphere and complete air tight containers

- Concerning fish oil emulsions specifically, the role of tocopherol and/or Vitamin E become influential due to higher ability towards prone to oxidation. On the same note, inert packaging becomes important too.
 - In conclusion to above, all of these remedies have been dealt with in the subsequent part of thesis to compensate these factors.
- Sodium oleate, as a co-emulsifier and/or stabilizer in the lecithin emulsions, have a pronounced effect by reducing particle size at higher pH values. It also has profound effect on the stability of the systems. Similarly, effect of sodium oleate in increasing the electrostatic repulsion between the droplets by increasing the absolute ζ - potential values was evidenced in the systems studied.
- Hydrolysis of lecithin (PC and PE) into its lyso derivatives along with aging time was proposed as the cause of decrease in pH values and increase in ζ - potential values of pH at 8 & 9 units. Decrease in pH during aging time has negative influence on the pH stabilized systems (i.e. IV emulsions)
- Buffer solution, if used, for the preparation of emulsions gave lower absolute ζ - potential values along with either very low stability or unstable systems. However, without using a buffer, IEP was calculated for approximately all the emulsion systems, which fell between 2 and 4 pH values.
- In terms of stability, S 45 and S 75 and H- 50 were considered appropriate at higher pH values, while R LPC 20 was considered optimum emulsifier if stability may be concerned along a large range of pH values. However, due to lyso- contents of lecithin in its ingredients, IV emulsions might not be preferable to prepare with such kind of lecithin.
- Stability related to egg lecithin (PL-90 and Lipoid E) have been dealt with separately above while systems in soy oil (equivalent to Intralipid®) have maximum stability for all formulations prepared.
- Subsequent part of thesis considered only basic pH values for the preparation of IV emulsions in the range as of the commercial products (8.3 to 8.7 units)

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Part III

**Development and characterization of novel parenteral emulsions with
new emulsifier systems of marine origin**

[Fresenius Kabi prospectus commercial products]

1. Novel Emulsifier Systems: Marine origin:

1.1 Introduction

Continuous development and innovation are the core values of products and technology that depends upon the ever increasing modernization demands for fulfilling complex applications. Concerning nutraceutical products with their formulation technologies, the scope of this innovation and development increases many folds due to the restrict environments and challenging nutritional demands of human body with acute complexity of the disorder. New emulsifier systems for parenteral emulsions are the self-motivation for the pharmaceutical or, more precisely, nutraceutical products. Fresenius Kabi, as one of the largest pharmaceutical companies of the world, invest tremendously on research and development of the novel pharmaceutical products to fulfill the market and increasing clinical demands in supplying up-to-date products. In this sense, Chemical Engineering department from Universidad of Huelva worked in collaboration with Fresenius Kabi to develop and characterize parenteral emulsions using new marine origin emulsifier systems to develop scientific approach towards qualification of novel nutraceutical products. This part of thesis is dedicated to the formulation and characterization of new parenteral emulsion products jointly designed by Fresenius Kabi and University of Huelva.

The designing and development of a pharmaceutical product along with its conformity through laboratory characterization is one aspect towards commercialization of a product, however, the regulatory part must be kept in mind and dealt separately after lab conformation. Clinical studies, animal studies and then its translation to human studies are the next steps before the launch of a commercial product. It is of great importance to keep the regulatory conformance of any new pharmaceutical product to be launched in the market. In this way, the scope of this study was to formulate and characterize the proposed products. The bureaucratic part related to the regulatory and product conformation is beyond the scope of this dissertation, however, Fresenius Kabi dealt with these regulations separately.

1.2 Emulsifier system: Food grade emulsifiers

By now, from the 2nd part of this dissertation, whereby use of lecithin based emulsifiers containing phospholipids for parenteral emulsions has been understood. Designing emulsifier systems for food/pharmaceutical grade products, i.e. parenteral emulsions, the regulatory aspect of the emulsions produced is more sensitive than any other industrial products. That is the reason why, extreme care has to be taken in designing a novel emulsion system. It has been also understood that an emulsifier must hold a polar and a nonpolar molecule within an emulsion system for sufficiently long time. The adequacy of the emulsifier along with its appropriate quantity defines the dynamics of the stability of that particular emulsion.

Emulsifier system essentially consists of an emulsifier capable of holding down the oil and the water molecule in their prescribed quantities sufficient enough for a prolonged period of time (McClements, 1999). The prolongation time as per pharma standards is most probably not defined in months, but more than a yearly duration, as per commercial demands of supply and shelf span. In this way, the type of emulsifier along with its adequate quantity is very important to decide an effective use in a commercial product. It is unworthy to comment here that a heterogeneous mix is also stable within few seconds after agitation; the optimization of the heterogeneity is to make the kinetic mix stable for a maximum/appropriate life span.

Secondly, an emulsifier system may contain a co-emulsifier that helps in emulsification along with the principle emulsifier. It has the quality to reduce down the surface tension on an oil/water interface, however, if used alone may not significantly affect the emulsification. Sodium oleate, in our case for the formulation of parenteral emulsions, was the co-emulsifier along with the phospholipids used. Sodium oleate not only helped in reduction of the interfacial tension between the two distinct molecules of oil and water but also in some cases controls the pH of the formulated system too. Likewise emulsifiers, co-emulsifiers were also used in very small quantities effectively. The choice of right co-emulsifier with its percentage along with the phospholipids was the goal for the optimization of product formulations. Sodium oleate as a co-emulsifier was used in parenteral systems for the current study. Use of sodium oleate for different mechanisms (stabilizer/co-emulsifier and pH adjuster) has already been discussed in emulsion systems by various authors (Christoph, 1998 and Werling et al., 2008 etc.)

Thirdly, stabilizers are also considered in food emulsions within emulsifier systems. However, they do not bear the same characteristics as of an emulsifier. Their purpose is not to hold down the oil/water junction but most stabilizers are high molecular weight biopolymers which have properties that increase the viscosity of the continuous phase or to modify the rheology of the aqueous phase, preventing the droplets to come closer and retarding creaming or flocculation (McClements, 1999 & 2005). Stabilizers also play a pivotal role in prolongation of the life of an emulsion along with emulsifiers used. They are large molecule polymers to induce stability in the already emulsified systems to hold down their structures for a larger period of time (e.g.

polyethylene glycol, PEG). There are many uses of PEG in the industry as it is non-toxic (Anderson, et al. 2007 & Sheftel, 2000) while having food grade acceptance by UK and US food authorities respectively (Food standard Agency, UK & Food and Drug Agency, USA). In pharmaceutical fields, PEG is used as an emulsifier and as a stabilizer too. PEG, Macrogol 400, (tested according to Ph.Eur., 400) was used as stabilizer in the current formulations system.

The most common polysaccharides used as stabilizers both in model studies or in commercial are xanthan gum, galactomannan gum, cellulose derivatives, carrageenan and pectin. Literature suggests that attempts have been made in their successful formulation and/or development of procedures of using these hydrocolloids to delay creaming. Huang et al. (2001), Lorenzo et al. (2012) and Moschakis et al. (2005) have based their research on gum arabic and xanthum guar with different and inline results depending upon the quantity of stabilizer used and the system of o/w emulsion in question.

1.3 Phospholipids: Emulsifier of animal/marine origin

Two different types of marine origin phospholipids were used to formulate parenteral o/w emulsions in this section. These novel phospholipids are basically marine oils, in principle, with characteristics of emulsifiers as well. Both emulsifier oils were sent by Fresenius Innovation and Development Center from Bad Homburg and were used in the current study as such without any further processing. The emulsifier oils used in this study would be called throughout this section as FKHEO30 and FKKO42 respectively. It was reported to have 30% total phospholipids content in FKHEO30 type emulsifier while 42% in FKKO42 type emulsifier, the rest of the composition was kept discrete as such. This part of the thesis shows the results obtained from the formulations prepared using different concentrations of FKHEO30 and FKKO42 phospholipids (emulsifier). This section also contains the optimization of the parenteral systems studied and in conclusion what systems are eligible as a preferable candidate for commercial product/s.

However, it is of great importance to note that the physicochemical characterization of these formulations is only one step towards commercialization of an industrial product and especially in the case of pharmaceutical products. To this extent, the scientific approach is only a door step towards commercialization and there are a lot of regulatory aspects to be taken into account before its commercialization. The approach here in this dissertation, with novel emulsifier systems, was to fulfill the scientific demand of the formulations prepared and make them presentable for further clinical/biological studies for regulatory purposes in the event for conformity.

In fact, pharmaceutical product design and formulations development is a very broad category theme with work efforts of a team with different origin, in reality. Here at University of Huelva, only scientific studies have been carried out to develop and optimize the product development and formulations. The regulatory part of this development is out of scope of this dissertation.

1.4 New emulsifier systems: Motivation of naturally occurring marine origin novel emulsifiers with enhanced characteristics and nutritional values

There is always a room for developing new emulsifier system and the motivation towards development of a new formulation is due fact to encounter more complex nutritional cases during and after disease cure, within same multi-purpose formulation. This helps to serve as not only a curing drug but also fulfilling nutritional demands of the body suffered during prolonged ailment effects. Suggested thoughts behind a novel emulsion system and hence, new emulsifiers are also aligned due to the provision of exhaustion of the already formulated systems and exploitation of new organic and inorganic substances for their multi uses within same frame line. To keep control of complex nutritional demands of the human body suffering prolonged ailment and designing multi-roles of active ingredients are generally the motivation behind developing novel emulsifying systems to formulate parenteral emulsions. This part of thesis focuses on formulating parenteral emulsions based on especially suggested marine based emulsifiers termed as FKHEO30 and FKKO42. These emulsifier oils, as raw nutrients, have never been used in emulsions as emulsifiers in any formulations. The advent of exploiting these oils with their dual properties as a food supplement as well as emulsifier was a unique characteristic that was exploited during this research work. Amount of phospholipids, around 30% and 42% respectively in FKHEO30 and FKKO42, as reported by the supplier, with an adequate percentage of phosphatidylcholine (PC) in its raw form, has made them in the category of naturally occurring PC like lecithin and its class. Lecithin emulsions have already been discussed in part II of this dissertation. Here in this part of thesis, different percentages of these oils (FKHEO30 and FKKO42) were used as emulsifiers along with different oil types in oil/water emulsions.

In order to study the emulsification process, the emulsifier properties are always an additional plus to explore and utilize for formulations in question. The study concerning o/w emulsions based on FKHEO30 and FKKO42 emulsifier systems is basically a preliminary research which will approach different aspects of its usage with different percentages and oil types. In this research work, information regarding visual analysis of emulsions along with its experimental characterization, based on which feasibility of considering the above mentioned parenteral emulsions as a commercial product will be discussed.

A typical visual observation is the presence of a physically formed characteristic layer due to the presence of this novel emulsifier at the top of each formulation independent of the emulsifier concentration (FKHEO30 or FKKO42) and type of oil used. This characteristic layer might not be confused to be as free oil drops and in this way, characterization of emulsion formulations formed by FKHEO30 was carried out independent of this layer and by all means reported too. This part also contains methods to reduce or overcome it, based on experimental evidences.

2. Materials and Methods:

This section deals with the materials used for the formulation of the emulsions using marine origin emulsifiers. Briefly a stepwise approach towards the materials used and the methodology adopted is as follows:

2.1 Aqueous phase

Millipore apparatus (Merck KGaA, Darmstadt, Germany) was used for milli-Q water with Millipore cartridge labeled SimPak®1. De-ionized water (DI) water controlled at pH 8.5 (sodium oleate addition or 1N NaOH solution) was used in all the formulations prepared in this section. Similarly, for zeta potential measurements, freshly extracted milli-Q water was used to have minimal effects of ions adsorption at water surface due to storage at lab ambient. Extreme care was taken in controlling/managing water quality, as aqueous phase contained 77-87% of the entire formulations, and a slight slip in water quality may give replication error in zeta potential values. Table 2.1 indicates different percentages of aqueous phases used in all formulations.

Aqueous phase also contains glycerol, (molecular biology grade, VWR Chemicals, Leuven-Belgium) with 2.2% (w/w) in all the formulations, the only exception was when a mixture of oils was used where the quantity used was 2.25% in those formulation. Glycerol was used as an osmotic agent in many parenteral emulsions as described by many authors (Klek et al. 2013 & Arie et al. 1999). Glycerol, being intensely hygroscopic in nature, was stored in airtight bottle and nitrogen gas purging was made after its use before closing the container, in order to guarantee its chemical purity. It was added in DI water when the temperature reached at 40-45 °C and after it, sodium oleate.

As indicated above, the aqueous phase was pH controlled with a range between 8.3-8.7 so, 1N base was used to serve this purpose by dissolving equivalent quantity of NaOH, (Panreac Quimica SLU, Barcelona- Spain and Sigma Aldrich GmbH, Steinheim- Germany) in milli-Q water. Sodium oleate was kindly sent by Fresenius-Kabi, Bad Homburg- Germany. Sodium oleate was preserved at -20 °C right after use, according to chemical safety instructions. Only 0.03% of sodium oleate was used in this study to enhance emulsification (as co-emulsifier) and in some cases, to maintain pH required as indicated above.

2.2 Emulsifier

As far as emulsifiers were concerned, in formulation systems studied, FKHEO30 and FKKO42 served this purpose and were kindly sent by Fresenius-Kabi from Bad-Homburg Germany. FKHEO30 contained phospholipids quantified from 30% max as indicated on the technical data sheet (TDS) provided by supplier while 42% phospholipids were present in FKKO42. It was sent with encryption from Fresenius Kabi and was used as such without purification and/or further treatment. The emulsifier oil storage was done with care and each time after use, the emulsifier was purged with nitrogen due to its opening and always stored at 5 °C in the fridge. Emulsifier oil was a part of oil phase for the said formulations.

2.3 Oil phase and oils: Fish, Soy, Olive, MCT and concentrated fish oil

Following types of oils have been used in this study. All oils are used separately for emulsion manufacturing, however; SMOF was used jointly as oil phase as indicated in Table 2.1. This table contains information on vitamin E and emulsifier oils too. Vitamin E as an antioxidant agent and emulsifiers were part of oil phase in emulsion formulation.

Table 2.1: Fresenius Kabi supplied oils and/or emulsifiers for the current research in the development and characterization of novel formulations.

Serial No	Article	Procured/Supplied from
1	FKHEO30 and FKKO42 (emulsifier oil)	Fresenius Kabi AG. GmbH
2	Omegaven (Concentrated Fish oil)	
3	Soy oil	
4	MCT (medium chain triglycerides)	
5	Olive oil	
6	Fish oil (refined)	
7	α tocopherol (Vitamin E- antioxidant)	

2.4 Methodology: Emulsion Preparation

Methodology of emulsions preparation has been dealt within the introductory part of this thesis in Part I. This section only refers to the preparation methods for the formulations discussed above. Here only a brief hint will be given for the methodology while the detailed section has been listed in part I.

The percentage of oils in respective emulsions for the formulations in question is presented in Table 2.2. This table is used as guideline for the said preparations and is held reference for any formulations preparations during this study in this part of dissertation.

2.4.1 Preparation Techniques

Preparation techniques for emulsions were almost the same as referred in Part I and II. However the idea was replicated from various preparation techniques for food based emulsions as discussed extensively by Tokle and McClements (2011), Nagasaka and Ishii (2001), Krickau et al. (2007), Knoth et al. (2005) and Choi et al. (2011) as indicated already in part II. Related to the preservative treatment, the protocol followed by Watrobska-Swietlikowska and Sznitowska (2006) was used in this research according to pharmacopeia standards.

Table 2.2: Formulation processing guideline with different percentages of oil, water and emulsifier phases.

Formulations	Emulsifier %	Oil phase %	Aquous phase %		
			Glycerol	Sodium oleate	DI water
1	FKHEO30 (0.6, 0.9, 1.2)	Soy oil (10/20)	2.2	Yes/No 0.03	Remainder
2		Fish oil (10)			
3		Fish oil + MCT(5+5)			
4		Omegaven (10)			
5	FKKO42 (0.6, 1.2, 1.8, 2.4)	Soy+MCT+Olive+Fish (6+6+5+3) α – tocopherol = 0.0135	2.5		

2.4.1.1 Aqueous Phase

DI water was heated with Agimatic E (J. P. Selecta S.A. Spain) having an electromagnetic agitator and temperature controller plate. The temperature was set to be at 60 °C with agitation no more than 500/600 rpm. PEG (macrogol 400) if necessary, was added at room temperature in aqueous phase. Glycerol was added when the temperature reached at 40-45 °C and waited for it to get dissolved.

Sodium oleate, if added in formulations was added at the same temperature (45 °C) and agitation was reduced to minimize the foam formation (see next section). Therefore, the temperature was let to increase till 60 °C. Testo 108 (Instrumentos Testo S.A. Barcelona- Spain) with a T-type thermocouple having range from -50 to +300 °C, was used to control temperature of this phase. At 60 °C, the aqueous phase was agitated for about 5/7 mins and then transferred to Ultra Turrax.

2.4.1.2 Oil phase

Seven different types of oils were used in this research work as indicated in Table 2.2. Percentage of these oils for respective formulations has been presented also in Table 2.2. Oil phase consisted of respective oil/s with emulsifier oil and vitamin E. The mixture was heated in a way that rapid heating was minimized but also heated efficiently so as to avoid degradation of oils at longer time exposure at higher temperatures. Temperature of the agitator was kept at 60°C and the oil phase was heated after aqueous phase to reduce the time of heated oil exposure to lab atmosphere (i.e. oxygen). When the mixture reached temperature around 55 °C, it was kept for 3-5 mins at the same temperature and then transferred to Ultra Turrax for pre-emulsion formation.

2.4.2 Coarse Emulsions: Primary homogenization

Preparation methods of coarse emulsions were explained in part I of this thesis. Briefly, Ultra-Turrax T 50 basic (Ika- Werke GmbH. Staufen, Germany) was used at 10,000 rpm to make 1000 gms (1L equivalent) of coarse emulsion samples at 55-60 °C (aqueous and oil phase) for a total of 10 mins. The coarse emulsions were checked and balanced with required pH value of 8.5 after processing.

2.4.3 Fine emulsions: Secondary homogenization

Secondary homogenization was carried out using a homogenizer Twin panda 600 (GEA Niro-Soavi, Parma- Italy) on pH controlled pre-emulsions for 6 numbers of cycle at 500 bars pressure. Method of fine/secondary homogenization is already described in Part I of this Thesis, for temperature and pressure adjustments of the different stages of the homogenizer.

2.4.4 Autoclave treatment

Sterilization was carried out for all the emulsions after homogenization treatment at a defined temperature of 121 °C with a time cycle of 15 mins [European Pharmacopoeia standard 5.0]. Steam autoclave AES-12 (Raypa S. A. Barcelona, Spain) was used to conduct sterilization at maximum pressure of 2 bars. Therefore, sequence of vial storage was followed for stability:

3 vials each for 25 and 40 °C while 1 vial at 5 °C (Total 7 vials stored at mentioned temperatures).

The characterization cycle was 1st and 2nd week stability check at 25 and 40 °C while after 4 weeks; stability check was made at 5, 25 and 40 °C. Worthwhile to comment again that due to no effect on the ζ - potential of the emulsions after this treatment, sterilization is carried out as presented by different authors in literature. (Schwarz and Mehnert, 1999 & Heiati et al., 1998).

2.5. Characterization

Characterization techniques involved visual, microscopic, particle size distribution analysis, osmolality, PFAT5 and zeta potential measurements. Density was also checked for the fresh sample and samples after 4 weeks of stability

Characterization discussion for the stability study of the samples prepared for this research work has already been discussed in Part I of this Thesis. For a detailed discussion on these techniques, reference is made to that part. Here in this section, only a brief note will be made in order to comment on each characterization technique.

2.5.1 Control of pH

Lab pH meter GLP- 21 with electrode 50 14 T (Crison S. A. Barcelona, Spain) was used to control and check the pH of formulations with a relative initial controlled value of 8.5 units. The protocol for measurement and balancing is described in part I as commented earlier in section 1, with reference to the acceptability criteria as explained in USP chapter <791> in relation to parenteral IV formulations.

2.5.2 Creaming test

For creaming evaluation, the protocol established by Roland et al. (2003) and McClements (2005) was followed. The appearance of characteristic emulsifier oil layer in emulsions, as discussed earlier (section 1), resulted in a special protocol and emulsions were not marked unstable if there was an evidence of that layer. Rather only naked oil drops were reported. A florescent lamp Waldmann (H2WC 111), kindly supplied by Fresenius Kabi, was used for visual observation and evidence was collected in the form of photographs for future reference during the stability study for 4 weeks. PFAT5 evaluation, see below, was basically the technique used to characterize any remarkable free oil contents in the formulations.

2.5.3 Particle size distribution (PSD)

The most important parameter in the stability and characterization of o/w emulsions is PSD measurement. USP <729> as indicated in Part I of this thesis, defines the acceptable size range of the largest particles in any IV formulations, i.e. 0.5 μm . Zetasizer Nano, series Zen 3600 (Malvern Instruments Ltd. Worcestershire. UK) was used to characterize the o/w emulsions. Only homogenized and autoclave treated samples were characterized for PSD measurement.

The protocol of measurement, replication and dilution has already been discussed in earlier part of this thesis and was followed as such for stability studies at different temperatures.

2.5.4 Zeta potential

Micro-electrophoresis instrument, Zetasizer with model Zen 3600- nanoseries (Malvern Instruments Ltd. Worcestershire, UK) was used to measure the ζ - potential from the principle of measurements of the electrophoretic mobility of the droplets in an applied oscillating electric field using laser Doppler velocimetry. Smoluchowski model was used to convert the mobility into ζ - potential automatically from the integrated software. In this study, protocol of Tantra et. al, (2010) has been followed for dilution of samples to avoid multi scattering effects.

The samples were diluted in their respective pH controlled solutions (8.5-8.7) and were filled in disposable folded capillary cells (DTS 1060, Malvern Instruments, UK) to relate zeta potential measurements and repeated with minimum three replications.

2.5.5 PFAT5 values

PFAT5 is a parameter to detect the free oil globule with a limiting value of acceptance within an IV formulation as defined by United States Pharmacopeia Chapter <729> as the "percentage of fat residing in globules larger than 5 μm (PFAT5) for a given lipid injectable emulsion [is] not to exceed 0.05%" (USP <729>). PFAT5 parameter was measured by Accusizer 780 APS from Particle Sizing Systems (Santa Barbara, California U.S.A), in the installations of Fresenius Kabi and the protocol of measurement has been defined in the primary part of this thesis as indicated earlier.

2.5.6 Osmolality

Osmolality is also discussed in first part of this thesis according to US Pharmacopeia “chapter <785>” that describes the regulatory aspects in conformity of a lipid emulsion. Vapro ® Osmometer, model 5600 (Wescor Inc, Logan, Utah USA) was used to measure the osmolality of the emulsions in the installations of Fresenius Kabi only and only for the formulations conforming PFAT5 values. The values from 288 to 310 mOsm/kg were considered within range as per USP <785>. The equipment was calibrated daily before every day use.

2.5.7 Density

DMA Analyser 4500 series from Anton-Paar (Graz, Austria) was used for density measurements having glass capillary tube at a calibrated temperature of 20 °C. Density measurements of freshly prepared autoclave sample was carried out in order to get an idea about the density of the formulation and how it deviated from the actual density of water at 1 gm/cm³. Protocol of density measurement was as described for the stability studies at different storage temperatures.

2.5.8 Microscopy

For microscopic analysis of any interesting emulsions, Olympus BX51 microscope (Olympus Corp. Tokyo, Japan) was used to analyze the formulations at a magnification of 50x fitted with an Olympus digital camera C5050. At this small magnification, it is obvious that a micro/nano emulsion drops cannot be seen, however, just to get brief idea, for all practical purposes, microscopic analysis was carried out to evaluate only free oil drops, if any.

3. Results and Discussion: New Emulsifiers for Parenteral Emulsions: FKHEO30

In this section, results from emulsions prepared by using novel emulsifier system, FKHEO30, will be discussed.

Emulsions containing oils phase with soy, fish, fish oil concentrates (omegaven and TG1 & TG2) and SMOF were used with FKHEO30 emulsifier oil mixture as indicated in the materials section. The water phase was kept the same with ingredients explained in materials section of this part. All emulsions were prepared with addition to oleic acid (0.15%) in the oil phase. Sodium oleate was not used as compared to the earlier formulations prepared with lecithin origin emulsifier in previous section. Also PEG 400 was used as a stabilizing agent for these emulsions.

Discussion on results obtained on formulation with FKHEO30 with different percentages and different oil types, as indicated above, is made in the subsequent sections of this part.

3.1 Percentage of FKHEO30, emulsifier oil, in formation of emulsions

Unlike lecithin in earlier formulations, where 1.2% emulsifier concentration was used as per references indicated in the materials section for the formulation of standard parenteral IV systems, (Tokle and McClements (2011), Nagasaka and Ishii (2001), Krickau et al. (2007), Knoth et al. (2005) and Choi et al. (2011)), FKHEO30 emulsifier was not significantly affected for the preparation of formulations with 1.2% concentration.

Rather, a variety of prepared IV emulsions formulated with 1.2% FKHEO30 emulsifier oil were not stable with higher concentration of this type of emulsifier. A clear instability was observed with approx. all the oils formulated with 1.2% or more FKHEO30 emulsifier with visible oil drops at the top. Experimental observation clearly indicated that fish oil and concentrated fish oils (omegaven, as per customized name from Fresenius) were separated apart into oil and water phases after autoclave treatment and hence the formulations were intendedly depleted with FKHEO30 emulsifier concentration to lower amounts (i.e. 0.9 and 0.6% FKHEO30 emulsifier). This case of instability was not observed for soy oil emulsions with 1.2% FKHEO30 and was considered for stability studies.

Formulations with equal to or more than 1.2% emulsifier were unsuccessful with the preparations so, the strategy was adopted within this section just to qualify this novel emulsifier system to be studied for stability studies to formulate with 0.9 and 0.6% FKHEO30 emulsifier oil (FKHEO30), apart from soy oil, whereby 1.2% emulsifier was also considered for stability.

In this way, subsequent sections in this part of thesis considers stability studies related to different oil types for the preparation of IV formulations using FKHEO30 emulsifier oil in concentrations of 1.2% (for soy oil), 0.9% and 0.6% respectively.

3.2 Percentage of oil loadings in emulsions containing FKHEO30 emulsifier

Due to the fact that the quantity of emulsifier, FKHEO30 oil, was not more than 1% in total in all the formulations studied in this part and also the total phospholipids available for emulsification in this type of emulsifier oil (FKHEO30) was 30% as such, as explained in materials section, the oil loadings were limited to 10% only for the systems studied (soy, fish and fish+MCT oils). Soy oil with 20% and SMOF, which also contained 20% oil mix in a ratio 6:6:5:3 (i.e. soy, MCT, fish and olive oil in respective ratios) were formulated but the results were in a complete separation of the emulsions after autoclave treatment. So 20% oil loadings, in general, were not appropriate for emulsification through FKHEO30 type of emulsifier.

Hence, soy oil, fish oil, fish oil with MCT and concentrated fish oil (omegaven) were considered in only 10% of emulsion loadings in the studies governing FKHEO30 emulsifier oil.

3.3 Emulsions containing 10% Soy oil with FKHEO30 emulsifier

In this section here, 10% soy oil emulsions prepared with FKHEO30 emulsifier oil will be discussed. As indicated in the materials section, oleic acid was used in these formulations and characterization was carried out in presence and absence of PEG 400. Results were compared with and without addition of PEG 400 for these formulations.

3.3.1 Characterization of 10% soy oil emulsions with FKHEO30 emulsifier

PSD, ζ – Potential, pH values and PFAT5 values of 10% soy oil emulsions prepared with FKHEO30 are presented in tabulated form in Table 3.1, with and without PEG 400 addition. The stability period was considered in reality with 5, 25 and 40 °C but failure to produce satisfactory stable emulsions, free of oil drops, the results are only presented for 25 °C here. Further, results after 4 weeks were unimportant to present due to lack of stability initially with the evidence of

the presence of free oil drops on top of these emulsions after preparation and autoclave treatment.

Table 3.1: Characterization of 10% soy oil with FKHEO30 emulsifier oil and oleic acid after processing in addition to PEG 400 and without its addition.

Oleic Acid with PEG/No PEG	Characterization after processing (1.2% FKHEO30)			
	PSD (nm)	ζ – Potential (mV)	pH	PFAT5
PEG addition	174	-70.2	8.67	0.569
Without PEG	177	-72.4	8.75	-
0.9 % FKHEO30				
PEG addition	171	-65.2	8.66	-
Without PEG	198	-63.8	8.69	-
0.6 % FKHEO30				
PEG addition	155	-75.2	8.88	0.525
Without PEG	197	-68.5	8.79	0.603

There were emulsions with 1.2% FKHEO30 emulsifier with PEG addition those values were the appropriate for characterization (i.e. PSD and ζ – potential) but PFAT5 evidence indicated the presence of free oil drops and hence the instability. Further, a thin emulsifier layer was also present on these emulsions.

Similarly, the formulations with 0.9% FKHEO30 also seemed stable but there were evidence of free oil drops after autoclave treatment. With 0.6 and 0.9% FKHEO30 emulsions, apart from free oil drops, there was a very small evidence of emulsifier layer worth negligible.

In this way, other than PFAT5 values and a visual evidence of very small and very tiny free oil drops at the top of each emulsions, the characteristics properties of emulsions indicated stability. But the evidence from PFAT5 values and the visual inspection from fluorescent light indicated free oil drops, so the emulsions were marked unstable. Results presented here are only for fresh preparation while study was completed in reality for 4-5 weeks of characterization. A typical microstructure of 0.6% FKHEO30 with PEG addition is presented in Figure 3.1.

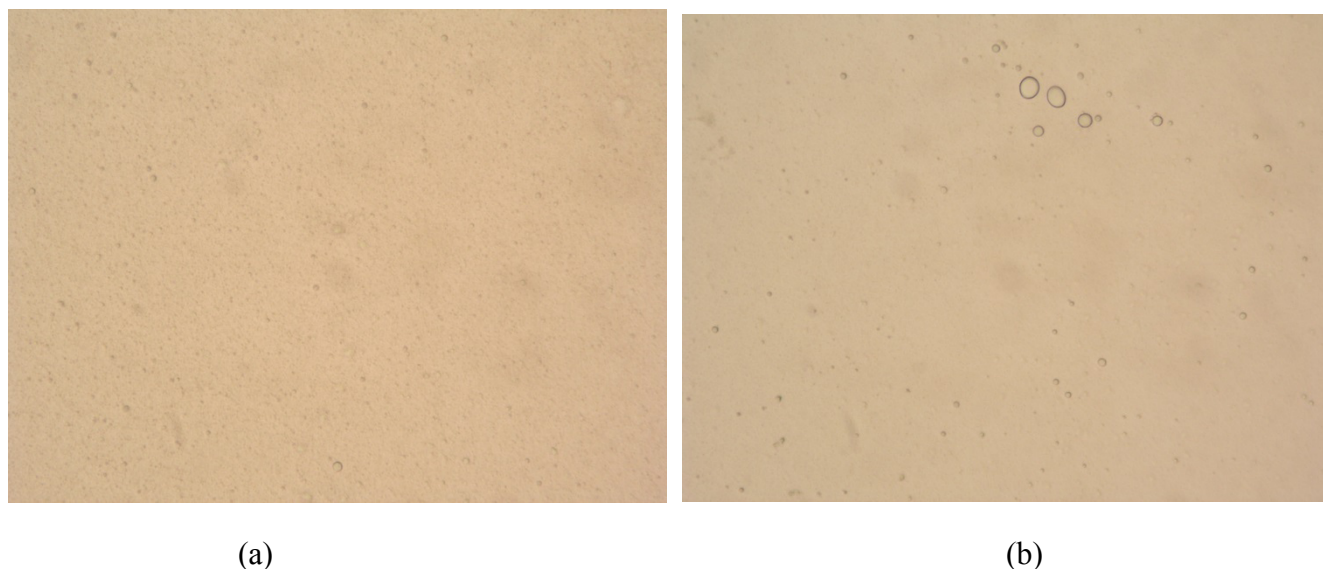


Figure 3.1: (a) and (b) Microstructure of soy oil prepared in 0.6% FKHEO30 with PEG 400. Visible oil particles were observed (at 50x).

3.4 Emulsions containing 10% fish oil with FKHEO30 emulsifier

The fate of emulsions containing 10% fish oil prepared with FKHEO30 emulsifier oil went through the same analogy as was with 10% soy oil and despite being seemingly ok, there were evidence of very small free oil droplets in the emulsions. Table 3.2 indicates the characterization values for PSD, ζ – potential, pH and PFAT5 (if any) for these formulations. Stability studies, though carried out through the respective temperatures (5, 25 and 40 °C), but not valued here due to the instability caused by free oil droplets and evidenced through PFAT5 results.

3.4.1: characterization of 10% fish oil with FKHEO30 emulsifier:

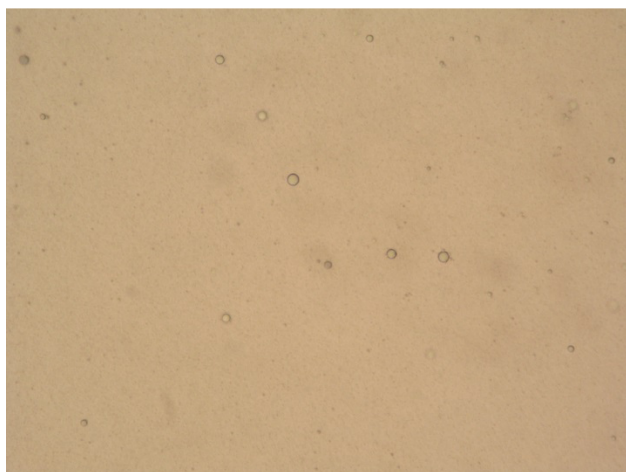
The results of characterization for different values are indicated in Table 3.2. PEG was added and compared to the emulsions without its addition.

As commented earlier, in section 3.3.1, the stability studies were held for 4-5 weeks but results here are presented for freshly prepared samples only due to evidence from the free oil observation in fluorescent light.

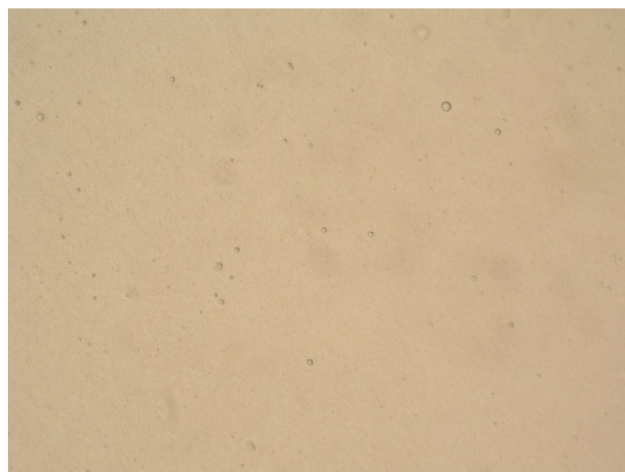
Similarly, microstructure of 10% fish oil with addition to PEG 400 and 0.6% FKHEO30 is presented in Figure 3.2, whereby visible oil drops can be seen in the micrographs attached.

Table 3.2: Characterization of 10% fish oil with FKHEO30 emulsifier oil and oleic acid after processing in addition to PEG 400 and without its addition.

Oleic Acid with PEG/No PEG	Characterization after processing (1.2% FKHEO30)			
	PSD (nm)	ζ – Potential (mV)	pH	PFAT5
PEG addition	-	-	-	-
Without PEG	197	-61.1	8.62	-
0.9 % FKHEO30				
PEG addition	198	-71.1	8.61	-
Without PEG	210	-69	8.47	-
0.6 % FKHEO30				
PEG addition	159	-58.3	8.0	0.568
Without PEG	176	-63.1	8.82	0.616



(a)



(b)

Figure 3.2: (a) and (b) Microstructure of fish oil prepared in 0.6% FKHEO30 with PEG 400. Visible oil particles were observed (at 50x).

3.5 Emulsions containing 5% fish oil + 5% MCT with FKHEO30 emulsifier

The emulsions containing 5% fish oil + 5% MCT prepared with FKHEO30 emulsifier oil had very good characteristics PSD and ζ potential values and despite being relatively looking ok, there were evidence of very small free oil droplets in the emulsions. Table 3.3 indicates the characterization values for PSD, ζ – potential, pH and PFAT5 (if any) for these formulations. Principally, PSD and ζ potential values were very appropriate but due to very tiny free oil drops, results are presented here only after fresh preparation. Stability studies, however, carried out through the respective temperatures (5, 25 and 40 °C), but not presented in Table 3.2 due to the instability caused by free oil droplets and evidenced through PFAT5 results.

3.5.1: characterization of 5% fish oil + 5% MCT with FKHEO30 emulsifier:

Table 3.3: Characterization of 5% fish oil + 5% MCT with FKHEO30 emulsifier oil and oleic acid after processing in addition to PEG 400 and without its addition.

Oleic Acid with PEG/No PEG	Characterization after processing (1.2% FKHEO30)			
	PSD (nm)	ζ – Potential (mV)	pH	PFAT5
PEG addition	193	-62.3	8.62	-
Without PEG	216	-58.1	8.55	-
0.9 % FKHEO30				
PEG addition	197	-63.1	8.52	-
Without PEG	203	-61.9	8.49	-
0.6 % FKHEO30				
PEG addition	187	-61.2	8.54	0.683
Without PEG	196	-60.2	8.44	0.705

3.6 Emulsions containing 10% concentrated fish oil (omegaven) with FKHEO30 emulsifier

Emulsions containing 10% omegaven prepared with FKHEO30 emulsifier oil containing 1.2% omegaven was separated after autoclave treatment, hence 0.6 and 0.9% FKHEO30 were prepared and was seemingly same as was with 10% fish oil and despite being characteristically ok, there were evidence of very small free oil droplets in the emulsions. Table 3.4 indicates the characterization values for PSD, ζ – potential, pH and PFAT5 (if any) for these formulations. Stability studies were though carried out for omegaven with 0.6 and 0.9% FKHEO30 emulsifier oil, yet the data is not presented here due to free oil drops presence. Studies with PEG were also held but the stability were again recorded very poor and no further results were reported after preparation.

3.6.1: characterization of 10% omegaven with FKHEO30 emulsifier:

Table 3.4: Characterization of 10% omegaven with FKHEO30 emulsifier oil and oleic acid after processing in addition to PEG 400 and without its addition.

Oleic Acid with PEG/No PEG	Characterization after processing (1.2% FKHEO30)			
	PSD (nm)	ζ – Potential (mV)	pH	PFAT5
PEG addition	-	-	-	-
Without PEG	196	-67.9	8.77	-
0.9 % FKHEO30				
PEG addition	211	-70.1	8.81	-
Without PEG	197	-71	8.78	-
0.6 % FKHEO30				
PEG addition	161	-64.8	8.44	0.616
Without PEG	213	-60.9	8.69	0.708

Figure 3.3 indicates microstructure of omegaven with PEG in 0.6% FKHEO30 with evidence of very tiny oil drops at 50x magnification.

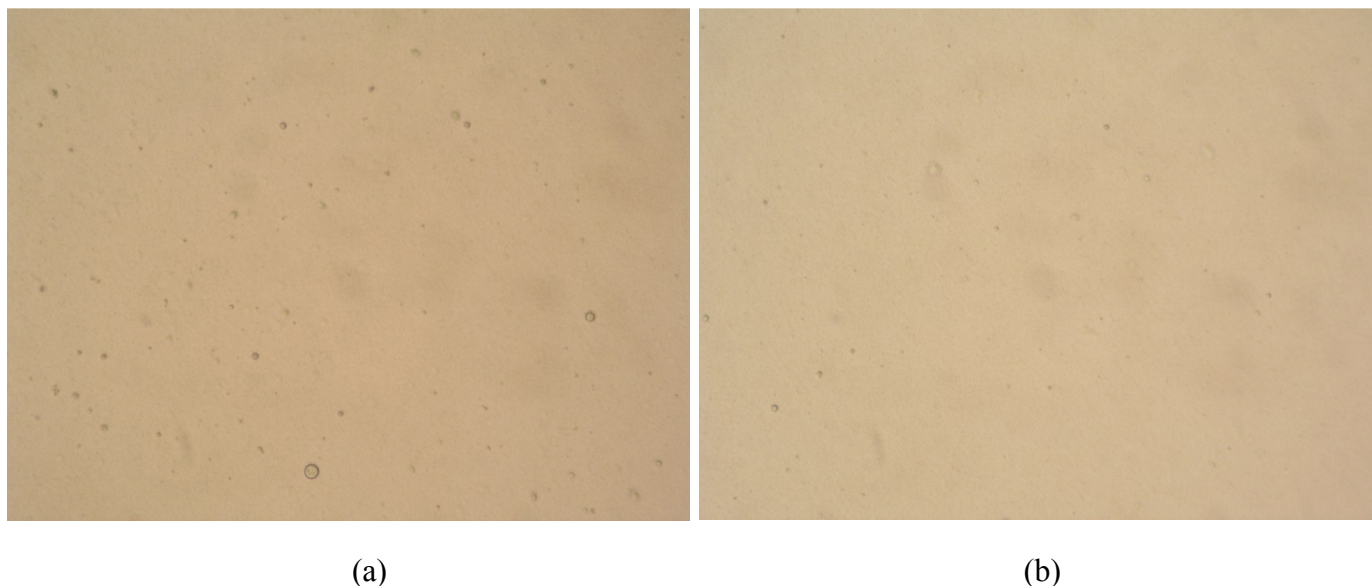


Figure 3.3: (a) and (b) Microstructure of omegaven prepared in 0.6% FKHEO30 with PEG 400. Visible oil particles were observed (at 50x).

3.5 Emulsions containing SMOF (20% loadings) with FKHEO30 emulsifier

It has already been commented earlier in section 3.1 that the oil loadings concerning FKHEO30 emulsifier oil were very limited due to the use of very low quantity of emulsifier, i.e. less than even 1%, the stability of formulations with 10% oil loadings were questioned rather to talk about a high oil loadings with the same emulsifier containing in total of only 30% phosphatidylcholine.

In this way, though emulsions were prepared with PEG 400 and in addition to oleic acid, there was no stability data associated to SMOF oil loadings due to their high oil contents.

The emulsions not only contained visible free oil drops at the top, there were evidence of emulsifier layer as well on top. Emulsifier layer was not a problem technologically and characteristically, as by agitation, it was removed. But the problem was presence of very clear free oil drops at the top of each formulation with 1.2, 0.9 and 0.6% FKHEO30 emulsifier oil concentration. Big oil drops were noticed after autoclave treatment and no evidence was required to collect from PFAT5 values too due to clearly seen in fluorescent light.

4. Results and Discussion: New Emulsifiers for Parenteral Emulsions: FKKO42

This section consists of the discussion on the results obtained with formulations using FKKO42 emulsifier oil with different oil types in sodium oleate addition and without its addition.

4.1 Soy Oil

Formulations were prepared using soy oil with three different percentages as indicated in materials section, i.e. 10, 20 and 30 percent. Sodium oleate was used primarily with the formulations and after that the stability study was also conducted without its addition.

4.1.1 Formulations having 10% soy oil with and without sodium oleate

FKKO42 was used with 0.6, 1.2, 1.8 and 2.4% with soy oil at 10% w/w concentration. Stability studies were carried out with respect to sodium oleate addition and without its addition. Amongst above mentioned FKKO42 oil concentrations, the 0.6% oil concentration did not give appropriate results and the emulsion was not stable with respect to physical appearance, as the oil droplets appeared (creaming) after autoclave treatment. Also, its particle size distribution had polydispersity and was marked unstable. The quantity of phospholipids in FKKO42 with its 0.6% usage in emulsion was not able to emulsify the water/oil interface properly and separation resulted. The color of the emulsion was very white contrary to other FKKO42 emulsions which were reddish in color as concentration of emulsifier oil increased. Similarly a related observation was the formation of characteristic oil layer which was not characterized as naked oil drops, rather a continuous very fine emulsifier layer which was dissolved by slight agitation in the emulsions, in ascending order of the usage of FKKO42 (more emulsifier, more oil layer). Also we can see in Figure 4.1, 4.2 and 4.3 related to 1.2, 1.8 and 2.4% emulsifier oil respectively, with and without sodium oleate addition, the PSD of these formulations during stability at different temperature and time (see section 4.4 and 4.5 respectively). In-process characterization and after a stability of 4 weeks, the particle size did not change so much. Also, the effect of sodium oleate was not very pronounced as compared to the formulations made with lecithin origin (see Part II). Both emulsion systems were stable containing sodium oleate and without it keeping emulsifier

oil percentage constant. While a trend in Figure 4.1, 4.2 and 4.3 was very prominent in the formulations that the more the percentage of emulsifier oil was the better the stability of emulsions with smaller PSD. Similarly, the formation and observation of emulsifier oil layer was more with more emulsifier percentage in the formulations.

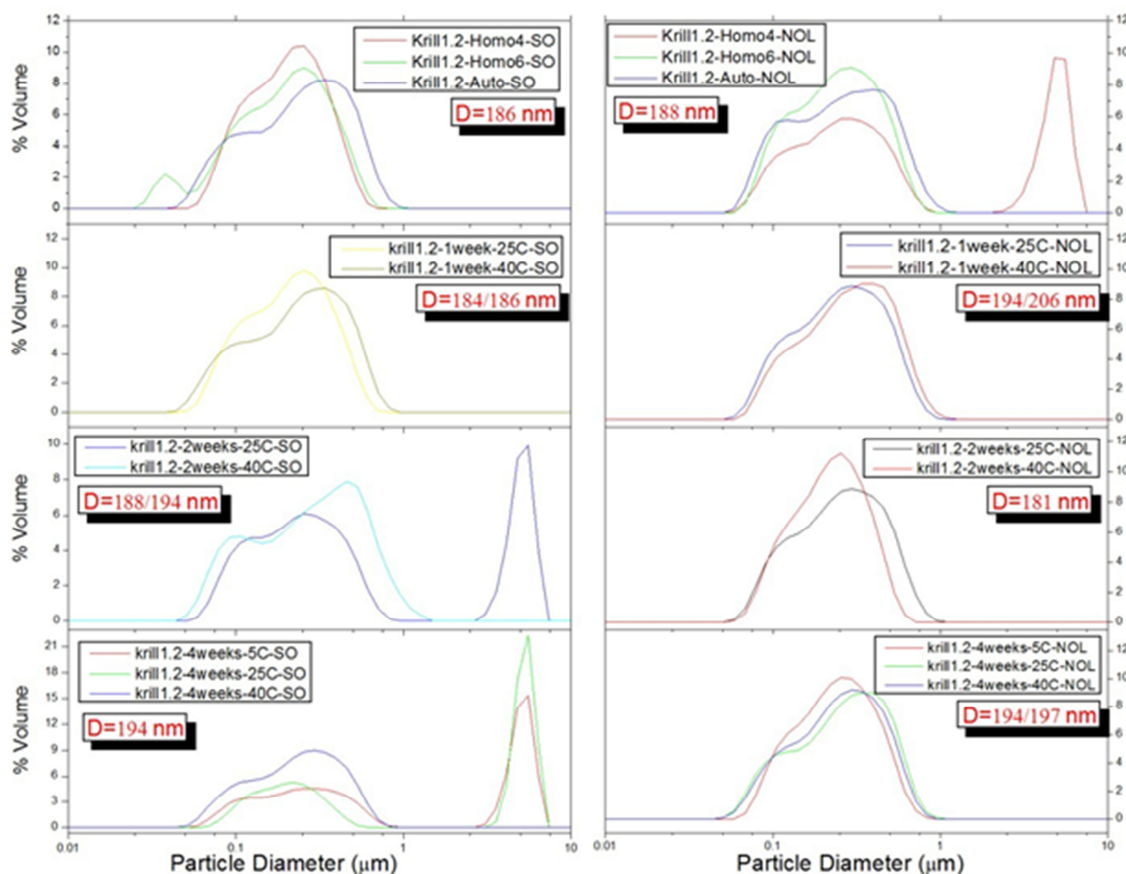


Figure 4.1: PSD of 1.2% FKFO42 oil in 10% soy oil formulations with and without sodium oleate addition at different temperatures along 4 weeks of storage. (legend SO is sodium oleate and NOL indicates no sodium oleate, while average particle size, D in nm, was indicated only once if observed same in a same graph).

By comparing the FKFO42 oil emulsions with three different percentages (independent of sodium oleate addition) that the particle size for 2.4% emulsifier oil added emulsion was the best as well as it showed suitable visual stability too (no creaming, no naked oil drops, no microbial growth and good overall appearance of emulsion). 1.2% formulation though indicated stability but their PSD and microstructure showed polydispersity (Figure 4.1) and clearly visible particles, respectively. So, 1.8% and 2.4% emulsifier oil emulsions were better than 1.2% FKFO42 oil due to availability of phospholipids in more concentration of FKFO42 when used in larger quantities.

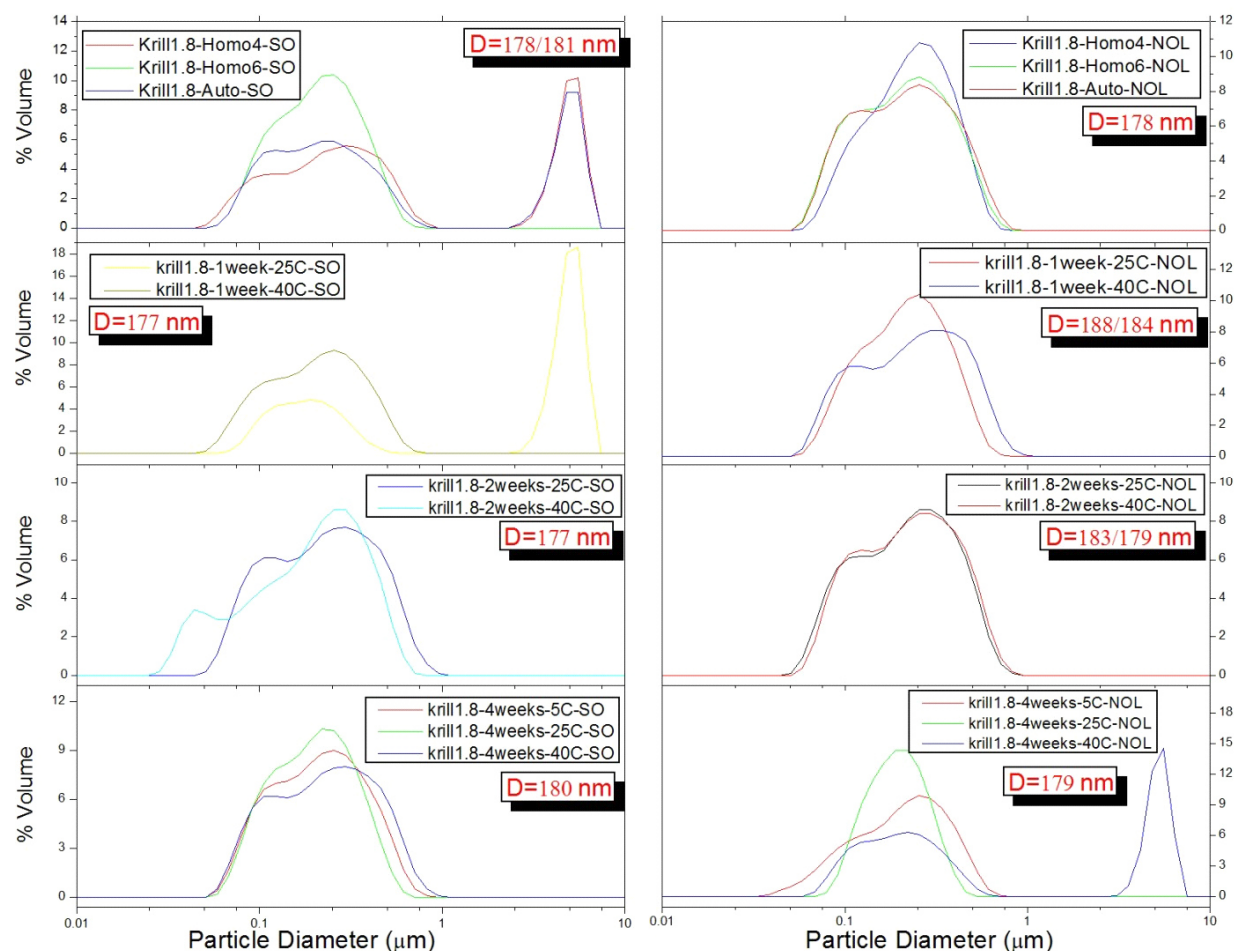


Figure 4.2: PSD of 1.8% FKKO42 oil in 10% soy oil formulations with and without sodium oleate addition at different temperatures along 4 weeks of storage. (legend SO is sodium oleate and NOL indicates no sodium oleate, while average particle size, D in nm, was indicated only once if observed same in a same graph).

Another observation in Figure 4.1, 4.2 and 4.3 was the descending order of PSD as emulsifier percentage increased, an appropriate and obvious observation related to the percentage of phospholipids present in FKKO42 emulsifier oil. Phospholipids are the emulsifiers with various origins as indicated and used by many research groups for parenteral emulsions due to their amphipathic character (Ishii et al., 1990; Ishii and Nagasaka, 2004; Ishii and Nii, 2005). Figure 4.1, 4.2 and 4.3 indicates that the PSD was decreased while using more phospholipid emulsifier percentage in the formulations indicating the availability of more phospholipids (equal to 42% in FKKO42) for emulsification with the same quantity of oil loadings used. The more the emulsifier concentration in the formulation was the smaller the PSD was obtained.

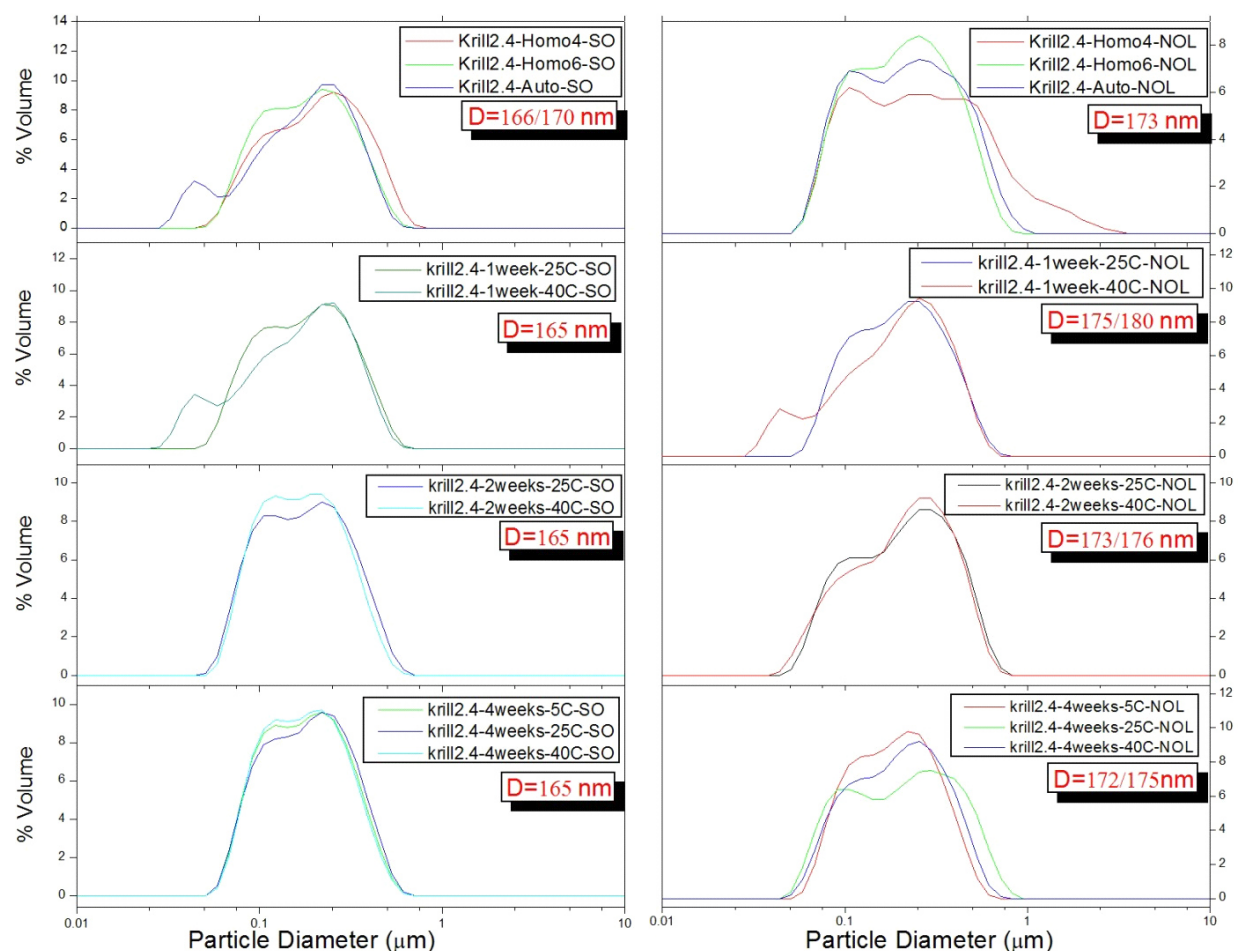


Figure 4.3: PSD of 2.4% emulsifier oil in 10% soy oil formulations with and without sodium oleate addition at different temperatures along 4 weeks of storage. (legend SO is sodium oleate and NOL indicates no sodium oleate, while average particle size, D in nm, was indicated only once if observed same in a same graph).

An overall 42% phospholipids in FKKO42 emulsifier oil indicated that FKKO42 comprises different phospholipids compound i.e. phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid etc (typical phospholipid constituents) as was the case with lecithin phospholipid discussed in Part II in reference to its heterogeneity (phospholipid constituents) as indicated by Washington (1990).

The heterogeneous mixture of phospholipids as constituents of an emulsifier enhanced emulsification process and increasing the percentage of FKKO42 emulsifier oil in emulsions increased the overall phospholipids and affected the emulsification during in-process and prolonged stability. Another very interesting observation related to the PSD of different percentages of FKKO42 oil formulations was the effect of time and temperature on the PSD curves, indicating no drastic increase or decrease in the median particle sizes. In Figure 4.6

ahead, it would be commented how pH changes with reference to time and temperature for the same formulations along with another physical entity i.e density. Density, however, was only calculated on fresh autoclave samples and after 4 weeks of stability, as indicated in materials section at all temperatures (5, 25 and 40°C), as indicated in section 2.

Zeta-potential measurements were very important in stability indication as per DLVO theory i.e. stable formulations result if its values fell out of ± 30 mV (Roland et al., 2003 and Rabinovich-Guilatt et al., 2004). Figure 4.4 indicates zeta potential measurements along time for the above mentioned formulations. The method of taking measurements was discussed in characterization section (section 2). Worth to indicate here that all zeta measurements were carried out at 8.6 - 8.7 pH values (pH controlled fresh DI water was always used for dilution).

Zeta potential values followed a typical symmetry for sodium oleate added 10% soy oil and formulations without its presence. Figure 4.4 is a typical graphical representation of ζ - potential values measured during the stability study for these formulations at different storage temperatures. ζ - Potential values for 1.8 and 2.4% emulsifier oil emulsions at 40 °C were mostly unchanged during the storage period studied, though they changed during 1 and 2 weeks' time but on 4th week, the final values were the same as were calculated for freshly prepared emulsions, independent of sodium oleate addition. Meanwhile, for the same emulsions (1.8 and 2.4% emulsifier oil), the absolute values of ζ - potential during storage at 25 °C, indicated an increasing trend for non-sodium oleate added formulations while the values did not change for sodium oleate added emulsions. The observation of ζ - potential (absolute) values for formulations at 1.2% emulsifier oil without sodium oleate addition, indicated an increase during storage time for 25 and 40 °C while they were decreased (absolute values) in the case sodium oleate was added at 25 and 40 °C. The storage at 5 °C, formulations followed another trend indicating no change for 2.4% emulsifier oil for sodium oleate and without its addition while at 1.2 and 1.8% emulsifier oil, all these emulsions showed decreasing (absolute) ζ - potential values during storage with sodium oleate and without its addition.

The reason behind it might be figured as the effect of low temperature suppressing hydrolysis reaction and hence the seizing any component of phospholipids transformation into its respective lyso- compound. Hydrolysis of phospholipids was a phenomenon extensively described by various authors (Werling et al., 2008) and indicated previously in lecithin systems and its affect was discussed in detail (Part II). Briefly, oil/water emulsion systems are always prone to hydrolysis due to an increased amount of water present as continuous phase and interaction of emulsifier and its phospholipid constituents might form hydrolysis compounds (e.g. lyso-). The lyso- compounds have more pronounced effect on ζ values as compared to its original phospholipid component (Jeong et al., 2001 & Chuah et al., 2009) due to negative ions presence.

This trend of changed/unchanged ζ - potential values at 5 °C may be postulated as an effect of temperature on the hydrolysis kinetics of emulsions during storage. A conclusion might be drawn with reference to this observation that lower temperature seizes the hydrolysis reaction and hence change in ζ - potential values. This postulate is discussed more deeply with evidence to further investigations during this study ahead on the basis of experiments with various oil loadings other than soy but with the same emulsifier oil (FKKO42) formulations.

At higher storage temperatures e.g 40 °C, the reaction rate/kinetics might be accelerated due to higher temperature but the data presented here so far for 10% soy oil emulsions, it may not confirm due to the systems studied only for 4 weeks' time. However, the zeta potential was either confined to the same values or it had increased for samples stored at 40 °C for most of the emulsions and same was the true for the samples at 25 °C.

Furthermore, the presence of naked oil drops on the top of 1.2 and 1.8% emulsifier oil, reported and mentioned in stability section of 10% soy oil formulations (4.1.2), may have caused a deviation in the similar trend for 5 °C formulations at 1.2 and 1.8% emulsifier oil as compared to 2.4% FKKO42 oil with no naked oil drops at top. The same trend may also be a cause of indifferent increase and decrease in ζ - potential values at higher temperature storage. In most of the cases, the characterization was resulted similar for both of the sodium oleate added formulations and without its addition. Emulsions prepared were stable with respect to DLVO theory if zeta potential values were considered criteria along with obtaining very low PSD.

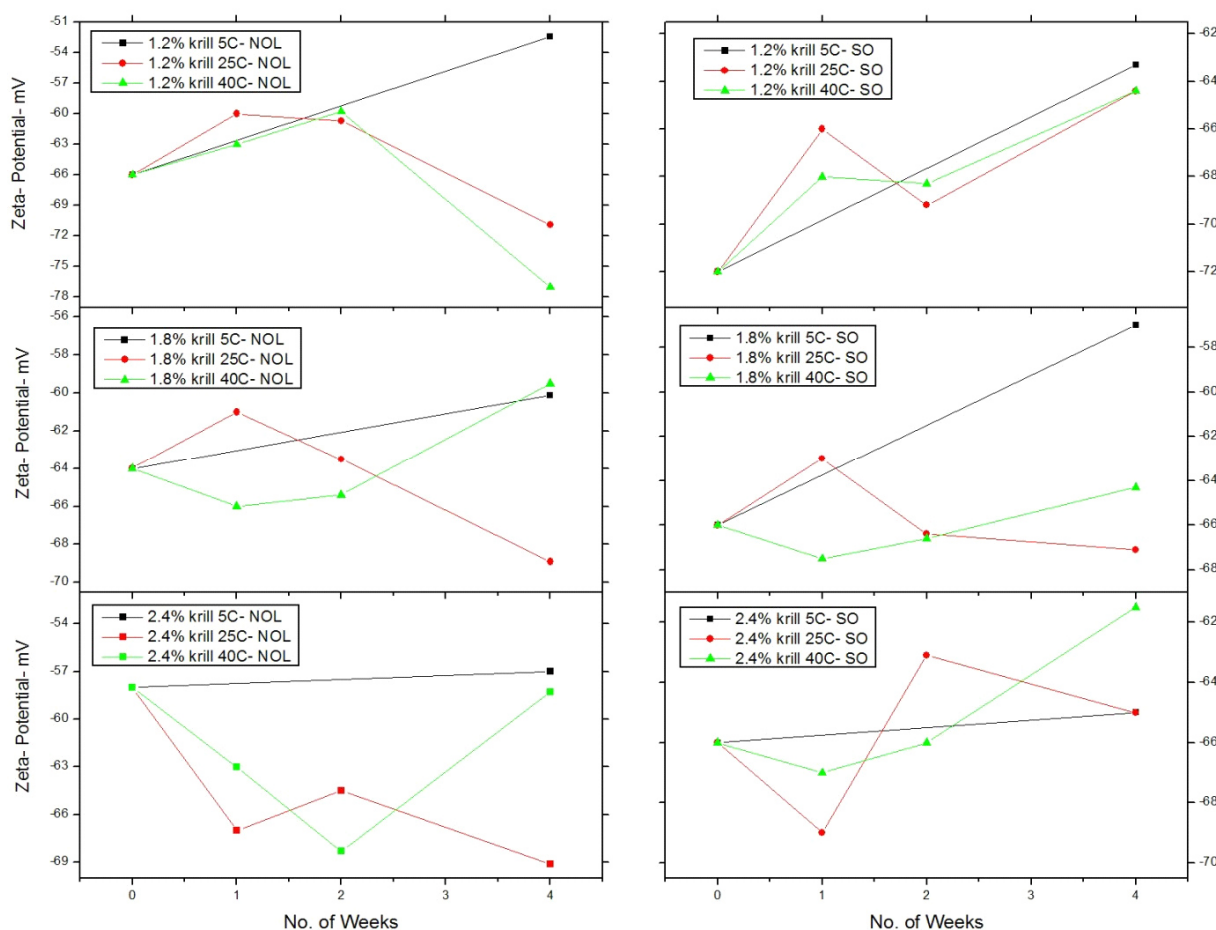


Figure 4.4: Zeta potential measurements of 10% soy oil and different emulsifier oil percentage with sodium oleate and without its addition at different temperatures versus storage time. (legend SO is sodium oleate and NOL is no oleate, "0 week" indicates fresh samples).

Density measurements at 20 °C did not indicate much difference from the initial values for freshly prepared samples and after 4 weeks of storage at different temperatures due to the fact that the systems were isolated and containing 80% water. Similar was the case with osmolality, and PFAT5 values and there was no significant change observed during the storage time and temperature. Also, sodium oleate addition and without its addition, the formulation characterization was unaffected. Table 4.1 indicates the average values of density, osmolality and PFAT5 characterization for the soy oil formulations (10%). Osmolality and PFAT5 values were carried out in Fresenius and are reported here as such.

Table 4.1: Characteristics measurement of 10% soy oil at various emulsifier oil concentrations measured after fresh preparation of samples and after storage of 4 weeks at different temperatures.

Storage time (weeks)	Storage temperature	Emulsifier oil Percentage (with/without Sodium oleate)		
		1.2	1.8	2.4
Density (g/cm ³)				
Fresh sample	-	0.9949	0.995	0.995
4 weeks	25°C	0.995	0.995	0.9949
Osmolality (mOsm/kg)				
Fresh sample	-	380	376	378
4 weeks	5 °C	378	381	380
PFAT5				
Fresh sample	-	0.04	0.021	0.018
4 weeks	40 °C	-	0.023	0.013

Similarly, for the pH values, Figure 4.5 shows the change in pH values during stability time and storage temperature. It was interesting to note that like zeta potential, the pH values also did not change much for the formulations stored at 5 °C from initial to final storage time (specifically for 1.8 and 2.4% emulsifier oil formulations). In fact, zeta potential and pH are inter-related as one can understand from the principle of measurement of zeta potential, as discussed in section 2 previously. An exception was 1.2% emulsifier oil emulsions, both with sodium oleate and without its addition i.e. it can be clearly see that the change in pH was more than what it was observed for 1.8 and 2.4% formulations. It has been stated already that 1.2% formulation was reported a stable emulsion, yet not an appropriate/ideal emulsion as compared to 1.8 and 2.4% emulsifier oil formulations. None of the emulsion rose to more than 9 pH value during stability study.

It can also be concluded that the function of a buffer was fulfilled by sodium oleate addition in formulations and as dictated earlier, it helped during in-process control of pH while during storage time at different temperatures (Christoph, 1998). Formulations even without sodium oleate addition withstood the same range of pH showing a good indicator towards emulsion stability and suppression of any by-product (such as lyso- compounds) during the stability period.

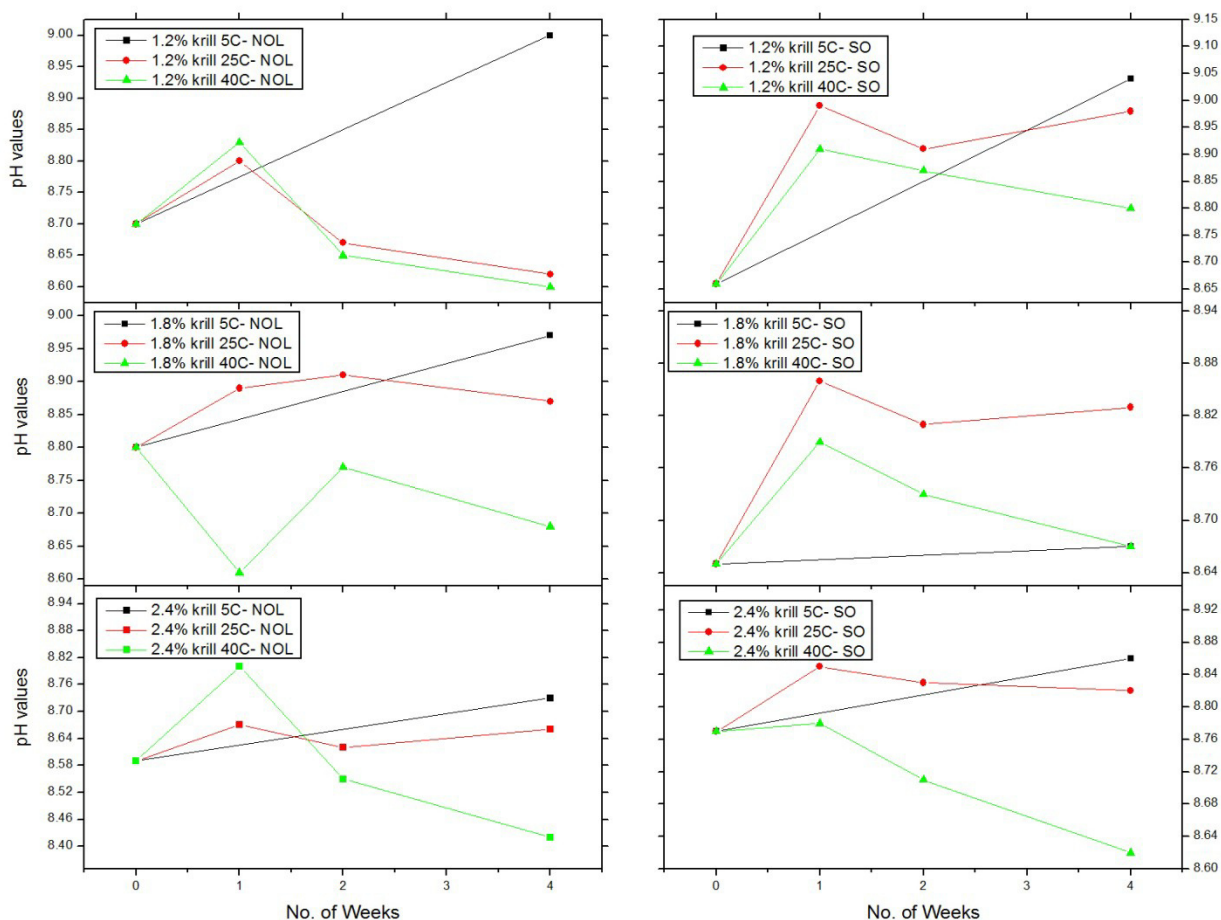


Figure 4.5: Change in pH values of formulations containing 10% soy oil with different percentage of emulsifier oil stored at different temperatures versus time. (legend SO is sodium oleate and NOL is no oleate, “0 week” indicates fresh samples).

Comparing Figure 4.4 and 4.5 for ζ - potential and pH values of the corresponding formulations relate in turns with zeta potential values as expected. It may also be observed that neither ζ - potential nor pH values for 1.8 and 2.4% emulsifier oil formulations during storage at 5 °C were changed indicating lyso- compound suppression due to temperature constraints. A reverse trend of increase in zeta potential value while decrease in pH for 1.2% emulsifier oil formulation might be justified due to the charging ability of naked soy oil drops present in the formulations during storage time as Hsu and Nacu (2003) suggested in discussion of the plateau region of ζ - potential at higher pH values. It should, however, be important to observe the corresponding zeta potential values of all the formulations measured in the stable range throughout the stability period studied corresponded in stable range as per DLVO theory ($> \pm 30$ mV).

4.1.2 Stability of 10% soy and FKKO42 oil emulsions:

Section 4.1.1 gave an idea about the stability of 10% soy oil formulations with 1.2/1.8/2.4% emulsifier oil during the discussion on PSD and zeta potential. Briefly, 1.2% emulsifier oil formulations indicated polydispersity in their PSD curves and after 4 weeks of, they had emulsifier oil layer at the top more enhanced than in comparison to 1.8 and 2.4% emulsifier oil emulsions.

The emulsifier oil characteristic layer also contained naked drops of soy oil at the top and the effect was shown in PSD due to its polydispersity (Figure 4.1). It should be noted that the addition of sodium oleate did not affect PSD curves and similarly, naked oil particles were observed on the top of these emulsions as well while distinguishing from the characteristic emulsifier oil layer. Smallest PSD was obtained with 2.4% FKKO42 with sodium oleate, as indicated in the PSD curves and evidenced in the micrographs presented in Table 4.2. However, a very small difference was observed in respect of 1.8% FKKO42 emulsions in comparison to 2.4% emulsifier oil emulsions. In this manner, a recommendation for the most favorable formulation in terms of stability would be 1.8% emulsifier oil formulation with sodium oleate addition, (better control of pH during processing) due to an optimum quantity of phospholipids usage in the emulsions. It was already discussed in Part II of this thesis as commented by Mirtallo JM, et. al., 2010, who compared 10% and 20% soy oil in 1.2% lecithin and concluded regarding the concentration of emulsifier in a formulation that free phospholipids become available when higher emulsifier to (drug) oil ratio was used indicating lipid related complications as was indicated by DAA manual, (2011) too.

Microscopic study using optical microscope at objective magnification of 50x revealed that there was no particle size changed during the stability period studied. Only few randomly selected micrographs are presented here for comparison purposes with different FKKO42 emulsifier oil concentration at different stability period and at different temperatures.

Images from fresh formulations, at 1.2% emulsifier, whereby visible oil drops were observed, 1.8 and 2.4% emulsifier concentration with no change at different temperatures and at different storage times are presented here. There was no difference with the formulations using sodium oleate and without its addition observed under microscope and hence it became unimportant to have comparison of them as such. Further, micrographs were cropped to their existing sizes without any further processing.

Table 4.2: Microstructure of 10% soy oil with different emulsifier oil concentration and sodium oleate (OL) addition, fresh and at different storage time and temperatures at 50x objective magnification.

1.2% FKKO42 with 10% soy oil- Fresh sample	2.4% FKKO42 with 10% soy oil- Fresh sample
	
1.8% FKKO42 with 10% soy oil- 5 °C- 4 weeks	2.4 % FKKO42 with 10% soy oil- at 40 °C- 4 weeks
	

4.1.3 Formulations having 20% soy oil without sodium oleate addition

Soy oil formulations with 20% concentration at 1.2, 1.8 and 2.4% FKKO42 emulsifier oil were studied for their stability evaluations only without sodium oleate addition, in this section. Discussion on the characterization of these formulations with respect of PSD, zeta-potential, microscopic analysis and density/pH evaluations will be presented.

Figure 4.6 and 4.7 shows droplet distribution curves of formulations without sodium oleate addition for 1.2, 1.8% and 2.4% emulsifier oil as a function of storage time. However, for comparison purposes only, sodium oleate formulations were also prepared and their zeta potential values were compared with that of formulations without sodium oleate addition. The polydispersity remains with time for 1.2 and 1.8% FKKO42 oil formulations with mean size ranges in the limits of 300 nm. The change in PSD with respect to storage time and temperature is almost negligible in these formulations as indicated in their respective figures

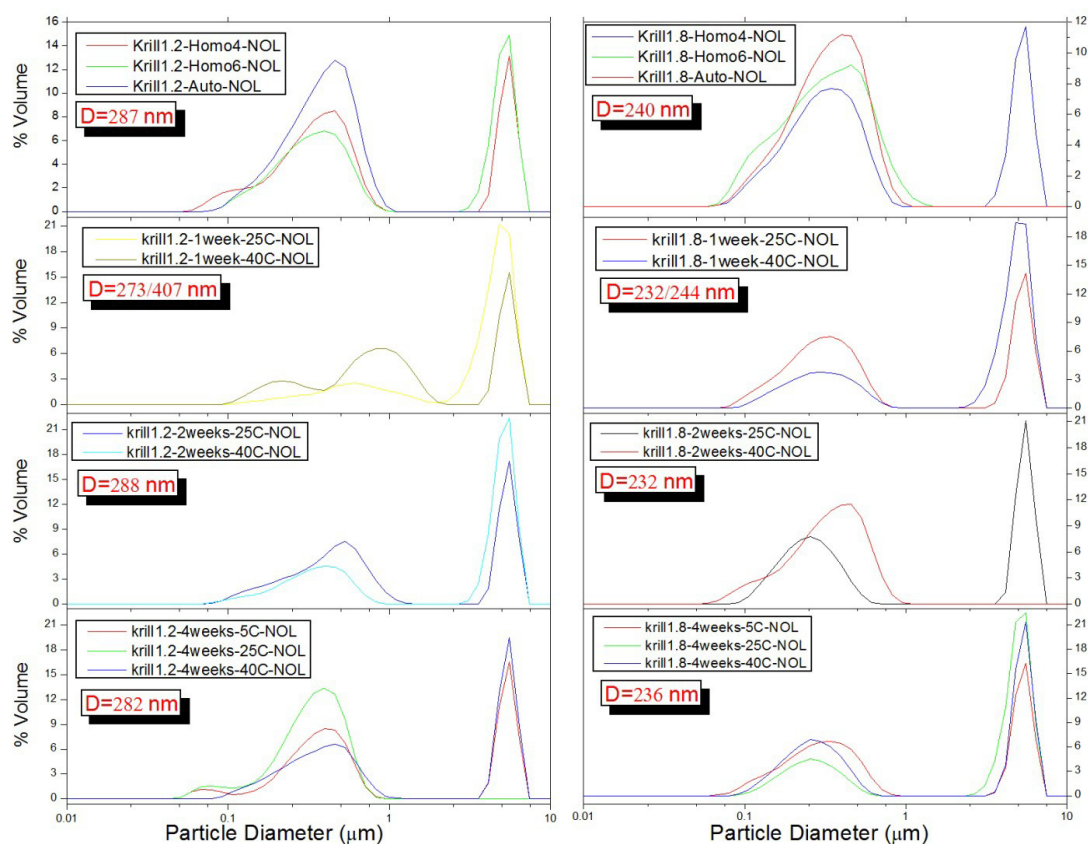


Figure 4.6: Evolution of PSD curves of 20% soy oil and 1.2 and 1.8% FKKO42 formulations without sodium oleate addition along time during different storage temperatures. (legend NOL is no oleate while average particle size, D, in nm, was indicated only once if observed similar in a same graph).

Formulations with 0.6% FKKO42 emulsifier were not prepared due to the poor stability results from 10% soy oil formulations prepared in section 4.1.1. As for as 2.4% FKKO42 emulsifier oil formulations (non- sodium oleate addition) were concerned, a low polydispersity throughout their storage time was depicted. Figure 4.7 indicates PSD curves of 2.4% FKKO42 with 20% soy oil formulations.

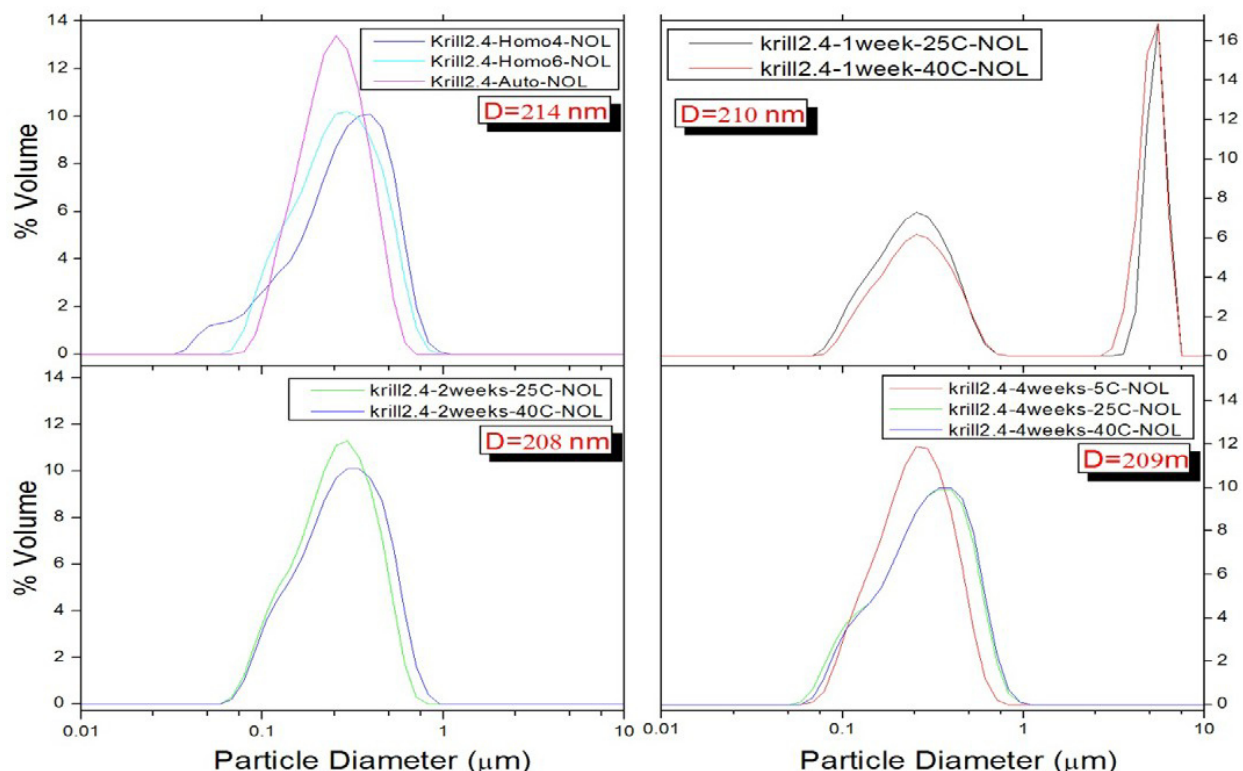


Figure 4.7: PSD curves of 20% soy oil and 2.4% FKKO42 formulations without sodium oleate addition along time at different storage temperatures. (legend NOL is no sodium oleate while average particle size, D, in nm, was indicated only once if observed similar in a same graph).

With reference to zeta potential measurements of sodium oleate added formulations, the highest value was measured for 2.4% FKKO42 oil equal to -72 mV which was also obvious due to its physical stability and after observing PSD in comparison to other formulations.

Similarly, zeta potential was measured for fresh samples and at different storage time and temperature and is presented in Figure 4.8 for formulations without sodium oleate additions. Figure 4.8 also contained change in pH values for different formulations along different storage time and temperature during the stability study.

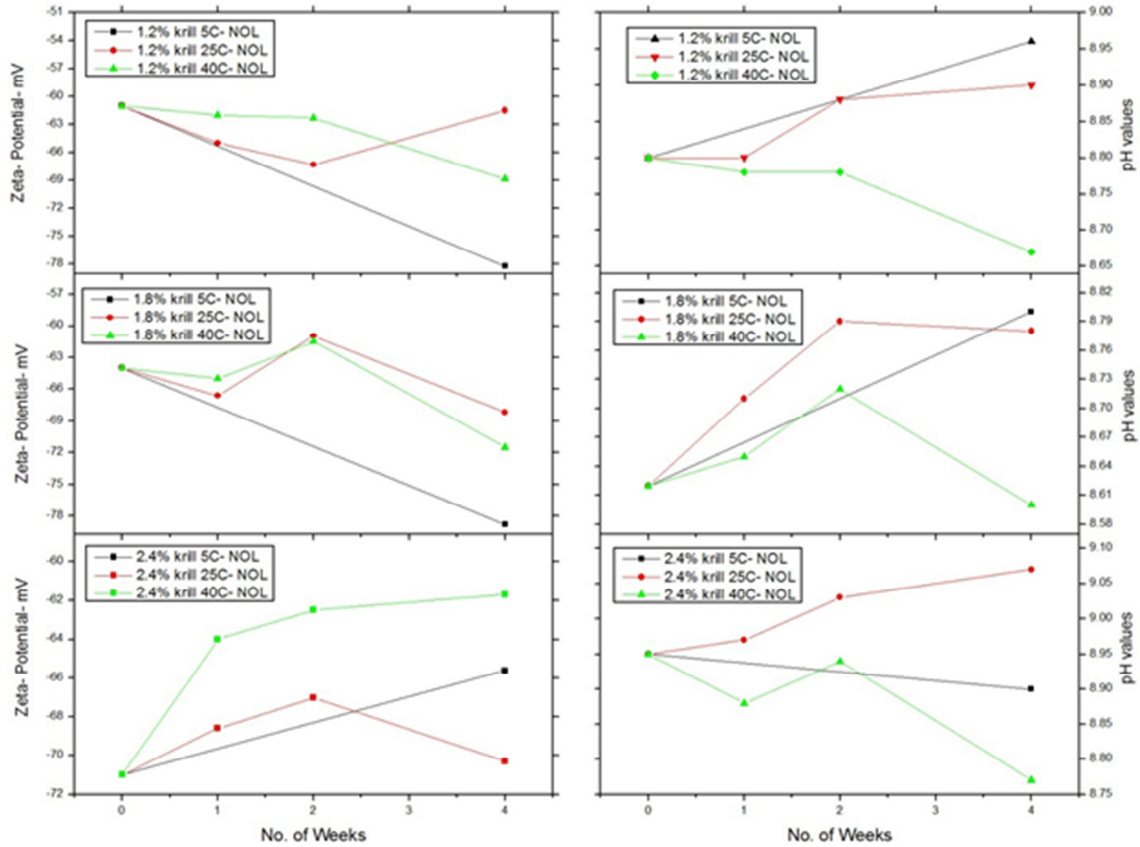


Figure 4.8: Variation of ζ - potential and pH values for 20% soy oil and different FKFO42 emulsifier oil formulations without sodium oleate addition (NOL) along time at different storage temperatures.

It can be clearly seen in Figure 4.8 that there is a direct dependence of ζ - potential and pH (both increasing) for 1.2 and 1.8% FKFO42 formulations as compared to 2.4% emulsifier oil formulation in storage at 5 °C during 4 weeks' time. Storage at 5 °C is important in terms of stability of emulsion at low temperature during storage after 4 weeks' time and its characterization would indicate a possible physical and/or chemical change (i.e. creaming and hydrolysis) in the formulation at lowest conservation storage period. Trends of change in pH and zeta potential for all formulations were almost similar and fell within their initial measured range after processing.

But for formulations at 5 °C, with 1.2% and 1.8% emulsifier oil indicated an increase in their zeta potential values (in absolute terms) and an increase in their pH values at 5 °C contrary to 2.4% emulsifier oil formulations, where a reverse trend was found, i.e. decrease in zeta potential (absolute) and a slight decrease in pH.

This fact has already been discussed regarding the characteristic hydrolysis reaction of o/w emulsion in Part II. At 5 °C, this reaction was suppressed due to temperature constraints and

could be the possible reason for the decrease in zeta potential (absolute) values for 2.4% FKKO42 formulations and for the decreased pH values too.

The analogy of the above fact had an argument based on hydrolysis reaction for 2.4% emulsifier oil, the question might arise for the same hydrolysis reaction at 1.2% and 1.8% emulsifier oil and at same storage temperature of 5 °C that why the zeta potential values increased in absolute terms?

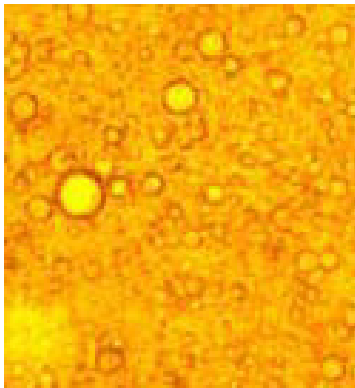
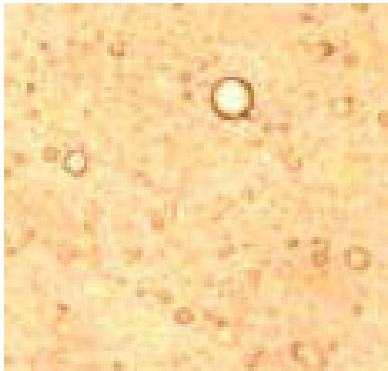
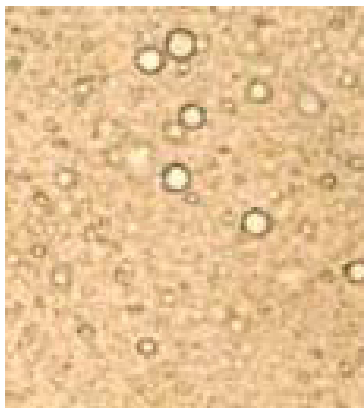
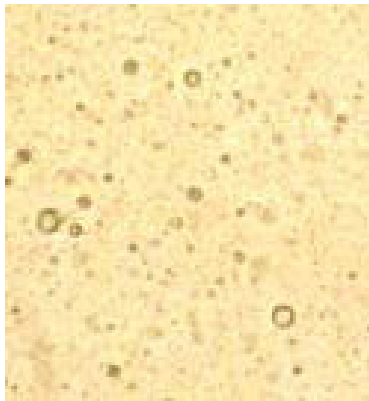
The answer to this question might be to the fact that on certain conditions (e.g, pH in aqueous media or soy oil impurities) the soy oil naked particles would be charged in emulsions along the storage time (Driscoll et al., 2001) and have affected the increase in zeta potential absolute values and hence the opposite trends. It will be discussed in section 4.1.4 for stability of 20% soy formulations where naked drops of soy oil particles were observed visible for these two formulations (Figure 4.5 and 4.6) while for 2.4%, the naked drops were quite less as might be depicted in for their PSD in Figure 4.7.

This trend was approximately similar for 25 °C and 40 °C for 1.2% and 1.8% FKKO42 formulations involving hydrolysis reaction products, lyso-phosphatidylcholine and other liberated lyso compounds, along with the charged naked soy oil particles (Werling et. al., 2008 and Driscoll et. al., 2001). Same argument holds true for 2.4% FKKO42 emulsion during storage at higher temperature. In short, a balance between the hydrolysis products, their suppression at lower temperature and charged naked oil drops would be the possible reasons for all these different trends in pH and zeta potential values for these formulations.

The density values of 20% soy oil with different FKKO42 emulsifier oil formulations without sodium oleate addition for freshly prepared samples and after storage at different temperatures for 4 weeks were similar throughout the period studied and were observed at 0.9864 gm/cm³. PFAT5 values were, however, out of limit from the USP standard and were recorded 0.052 to 0.061 for 1.2 and 1.8% FKKO42; while it was recorded 0.046 for 2.4% FKKO42 emulsions for fresh samples. Osmolality was not measured for 1.2 and 1.8% emulsions while 2.4% formulations gave 423 mOsm/kg average values for fresh samples.

Images of micrographs taken at 50x magnification for selected non-sodium oleate added formulations for fresh and at different storage time and temperature were indicated in Table 4.3. 1.2% and 1.8% FKKO42 formulations have clearly visible particles at all temperatures and storage times while for 2.4% formulation, the microscopic images were not so clear due to the reduction of droplet size.

Table 4.3: Microstructure of selected 20% soy oil with FKKO42 emulsifier oil formulation without sodium oleate (NOL) addition freshly prepared and at storage time during stability.

1.2% FKKO42 with 20% soy oil- Fresh sample	2.4% FKKO42 with 20% soy oil- Fresh sample
	
1.8% FKKO42 with 20% soy oil- 5 °C- 4 weeks	2.4 % FKKO42 with 20% soy oil- at 40 °C- 4 weeks
	

The evaluation of stability for 20% soy oil with different FKKO42 emulsifier oil formulations was clearly determined due to the fact that for 1.2 and 1.8% emulsifier oil emulsions there were clearly visible oil drops seen at the top throughout the stability period. For 2.4% emulsifier oil formulations, it was difficult to observe the difference between the naked particles and the characteristic emulsifier oil layer. PSD curves and zeta potential evaluation helped in characterization of 2.4% formulations and it was characterized stable for the stability criteria. In this way, 2.4% FKKO42 emulsions were found appropriate for 20% soy oil formulation and hence the only formulations to fulfill stability criteria along time and at different temperatures.

4.1.4 Formulations having 30% soy oil with and without sodium oleate addition

Considering formulations with 30% soy oil with FKKO42 emulsifier oil, the quantity of emulsifier was not enough to hold down the oil/water interface to a stable time span. In this way, 1.2% and 1.8% FKKO42 emulsions were inadequate as there were free oil drops observed at the top after autoclave treatment as indicated in Figure 4.9.



Figure 4.9: Autoclave treated vials containing naked and huge oil drops for 30% soy oil and FKKO42.

Similarly, the mean droplet size of the formulation using lower concentration of FKKO42 emulsifier oil was very high for this type of systems (with 30% soy oil). The reason could be translated as 30% loadings were too high for a formulation with 1.2/1.8 or 2.4% emulsifier oil and were all fell in an unstable range. The effect of sodium oleate addition on the emulsions was negligible as depicted in the PSD of respective emulsions.

Figure 4.10 indicates the PSD curves obtained after characterization of FKKO42 emulsions in 30% soy oil. It can be clearly seen from the PSD curves that the particle size was in a higher range for even sodium oleate added emulsions falling in the range of 300-400nm for 1.2 and 1.8% and for 2.4% emulsifier oil, this range shortens to 200-300 nm, however, there were evidence of polydispersity in the PSD as indicated in Figure 4.10. Alongside, naked oil drops were also observable at the top along with emulsifier oil layer.

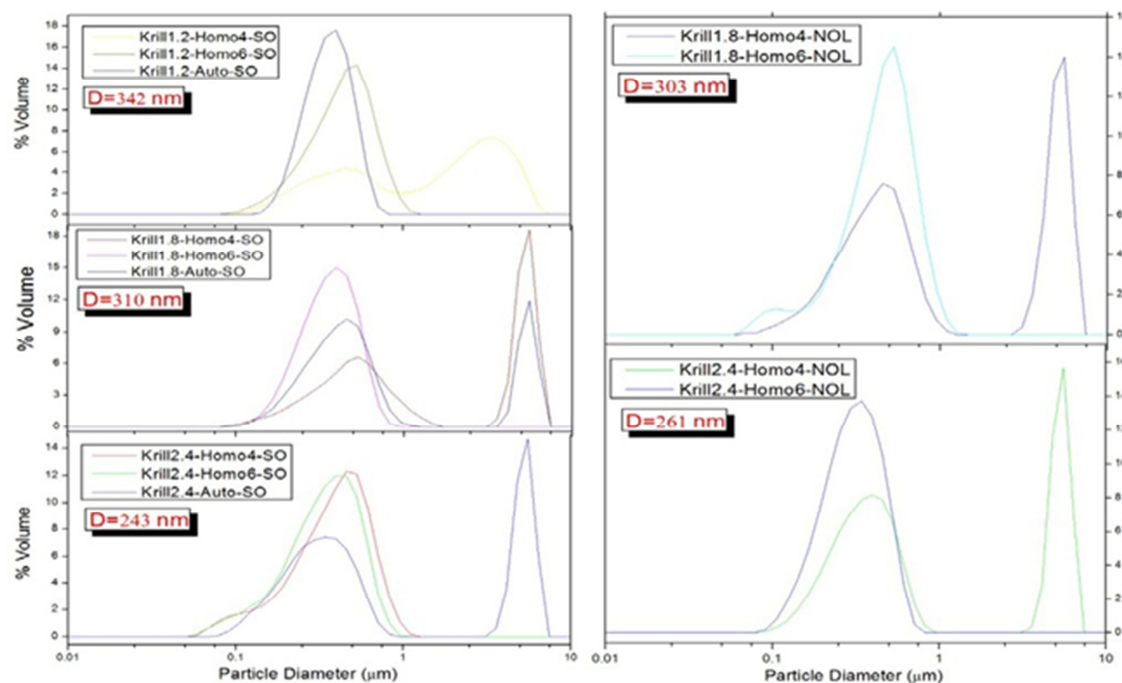
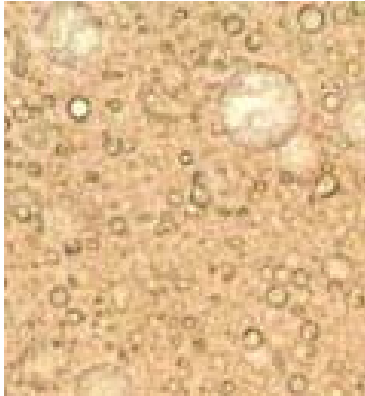
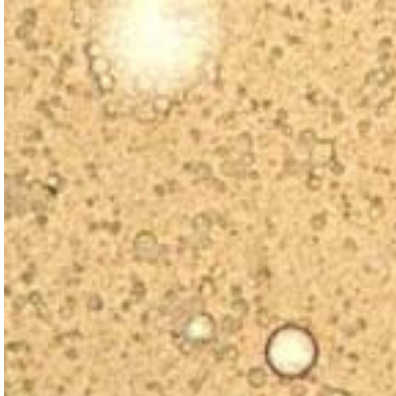


Figure 4.10: PSD curves of 30% soy oil with 1.2/1.8/2.4% FKKO42 emulsifier oil formulation along sodium oleate and without its addition. (legend SO is sodium oleate and NOL is no sodium oleate while only autoclave treated sample particle size is displayed on the graphs).

Density values were measured 0.997 for 2.4% FKKO42 emulsions, no PFAT5 or osmolality measurements were done due to visible oil drops. However, ζ - potential values were in the range of -70 for 2.4% FKKO42 emulsions. Microscopic images, as indicated in Table 4.4, show large globules of free oil in the emulsions.

Table 4.4: Images of micrographs taken for 30% soy oil with 1.8 and 2.4% FKKO42 oil formulations without sodium oleate addition.

1.8% FKKO42 with 30% soy oil- autoclave treated sample	2.4 % FKKO42 with 30% soy oil- autoclave treated sample
	

4.2 Fish Oil and its concentrate

In presence of different percentages of FKKO42, refined fish oil and its concentrate along with a controlled quantity of medium chain triglycerides (MCT) in emulsions have been formulated subsequently. Firstly, the results about refined fish oil will be discussed then mixture of fish oil with MCT and in the end of this section formulations with fish oil concentrate will be discussed. The percentage of fish oil and/or MCT was conserved at 10% in these formulations as discussed in materials section (Section 2, Table 2.2).

4.2.1 Formulations having 10% refined fish oil with and without sodium oleate addition

The first type of fish oil formulation prepared and studied for stability was 10% fish oil with 0.6/1.2/1.8 and 2.4% FKKO42 emulsifier oil in presence of sodium oleate. Fish oil used in this study contained 13% EPA and 9% DHA while total quantity of omega-3 acids were recorded as 28% as per certificate of analysis provided by the supplier (Fresenius- Kabi GmbH). Following are the results of PSD obtained with fish oil emulsions formulated with different percentages of FKKO42 emulsifier.

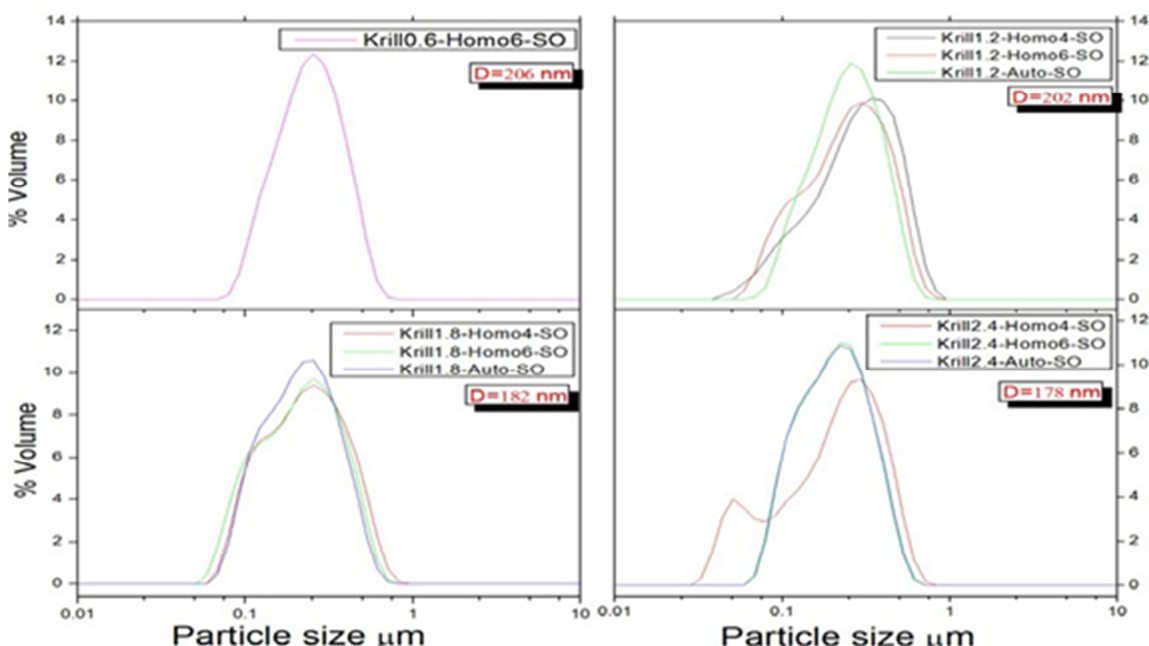


Figure 4.11: PSD curves of sodium oleate added 10% fish oil formulations with different FKKO42 emulsifier oil (legend SO is sodium oleate and average particle size D, in nm, is written for autoclave treated samples only).

A repetition was made without sodium oleate addition for the same formulations indicated above, except for 0.6% FKKO42 emulsion, which had not sufficient emulsifier quantity and was unstable. Figure 4.11 shows the PSD curves of freshly prepared 10% fish oil emulsions with FKKO42 and sodium oleate addition.

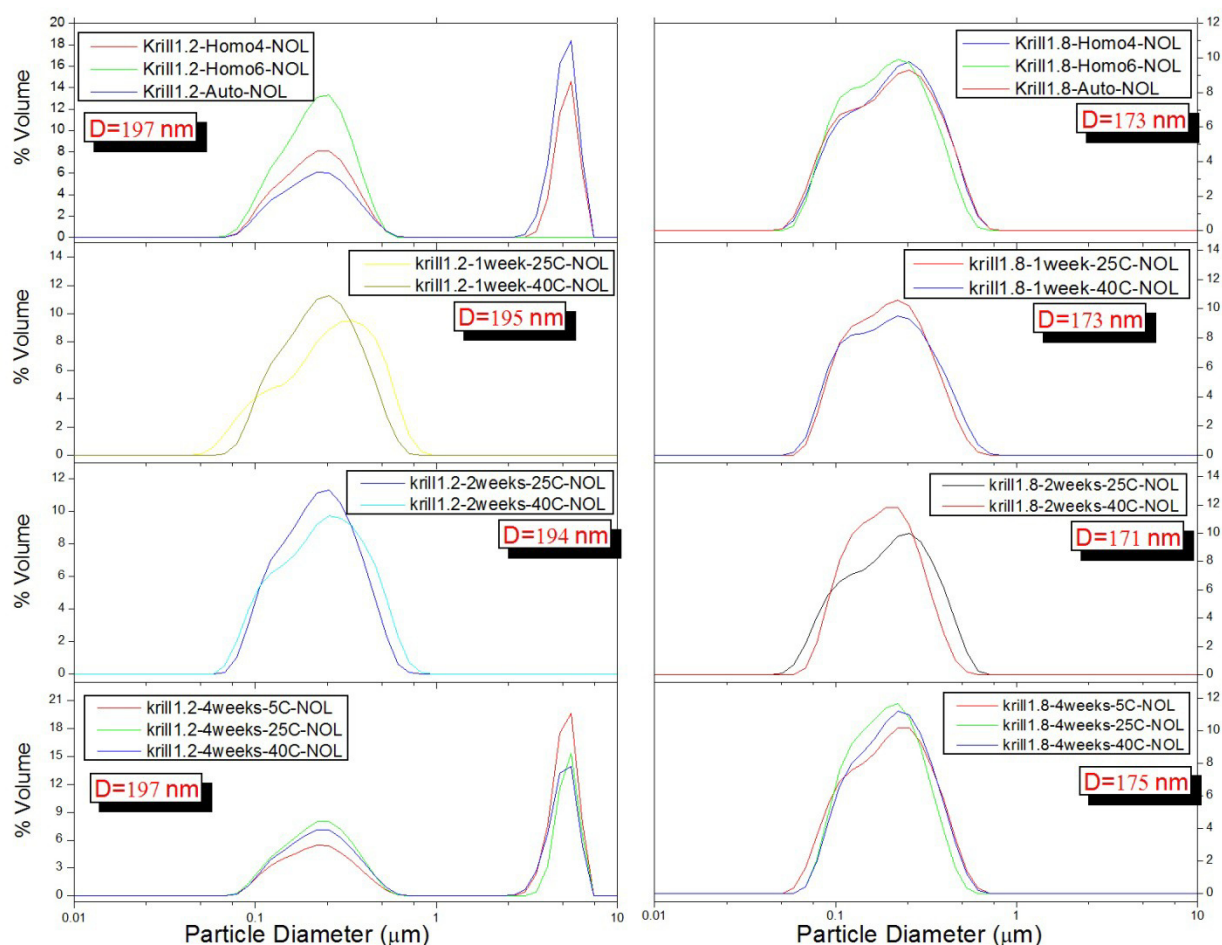


Figure 4.12: PSD curves of 10% fish oil and 1.2/1.8% FKKO42 formulations without sodium oleate addition along time during different storage temperatures (legend NOL is no sodium oleate and average particle size D, in nm, is written for smallest sized samples only).

Physical appearance of 0.6% and 1.2% FKKO42, sodium oleate added formulations were not identical as compared to 1.8 and 2.4% FKKO42 formulations, where latter were stable with no oil drops at the top. 0.6% formulation was having naked oil drops at the top, though no polydispersity found in its PSD curves yet after autoclave treatment, the separation of oil drops were clear. Both 1.8% and 2.4% FKKO42 formulations showed a very fine distribution of particle size ranging below 200 nm. These systems were studied without oleate addition at different stability weeks' time at different storage temperatures.

Figure 4.12 and 4.13 consist of PSD of formulations without oleate addition at various parameters studied along time. Similar to Figure 4.11, a very fine particle size range was also observed in PSD curves of 1.8 and 2.4% formulations without oleate addition indicated in Figures 4.12 and 13. 1.8% FKKO42 emulsions fell in 170 nm range while 2.4% emulsions fell in 160 nm particle size range. 1.2% FKKO42, however, gave somewhat polydispersity, a typical characteristic of 1.2% formulations observed throughout this study.

From Figure 4.12, 1.8% formulations were more appropriate than 1.2% FKKO42 formulation with particle size range indicated earlier. The formulations containing 1.8% FKKO42 emulsifier oil with 10% fish oil with and without sodium oleate addition was an optimum choice if stability and PSD were concerned. Figure 4.13 shows PSD curves for 2.4% FKKO42 emulsions too, indicating a characteristic narrow range of particle size (165 nm). In this way, it might be recommended along with 1.8% FKKO42 emulsions that the formulation with 2.4% FKKO42 was also of an optimum choice. Sodium oleate addition is always a plus due to its better in-process control on the pH of formulations as stated in earlier (Christoph, 1998). For an idea on the pH of the formulations during time and storage at different temperatures, Figure 4.14 explains it briefly.

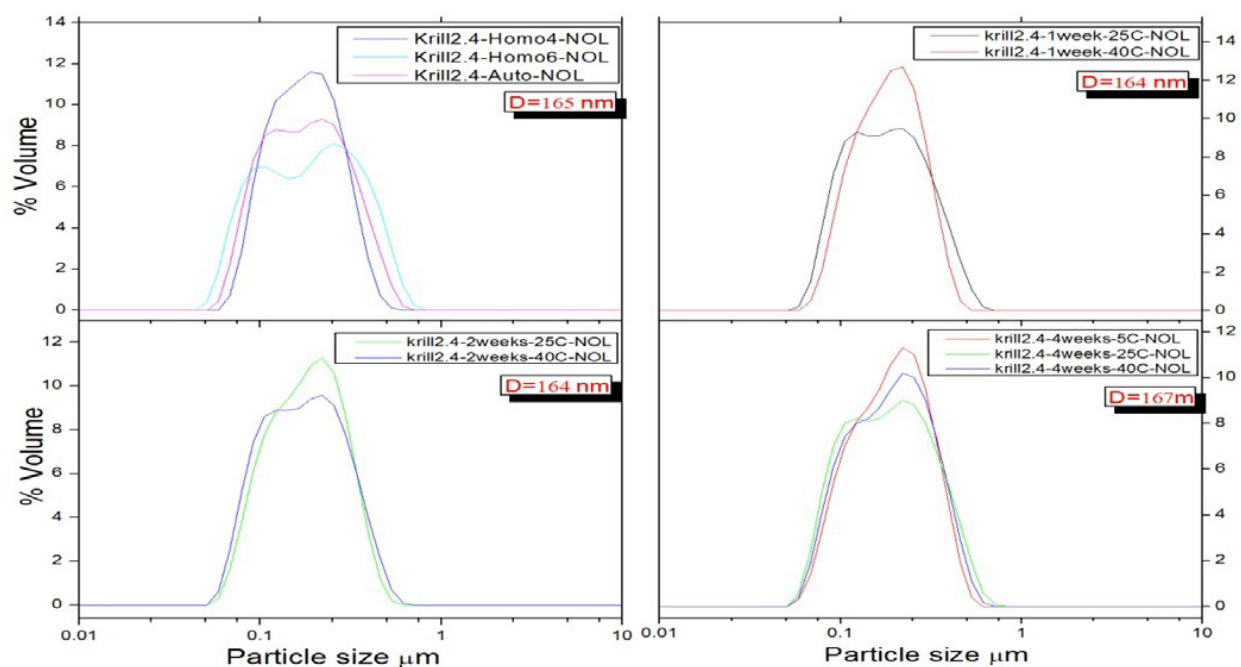


Figure 4.13: PSD curves of 10% fish oil and 2.4% FKKO42 formulations without sodium oleate addition along time during different storage temperatures (legend NOL is no sodium oleate and average particle size D, in nm, is written for smallest sized samples only).

Zeta potential of 10% fish oil with sodium oleate added formulations fell in -58 to -62 mV range for all formulations. The pH and zeta potential values are shown in Figure 4.14 for formulations

prepared without sodium oleate. The change in trend of pH and zeta potential at different storage temperatures along time with different FKKO42 emulsifier oil formulations is obvious in Figure 4.14 showing trends particular to these formulations.

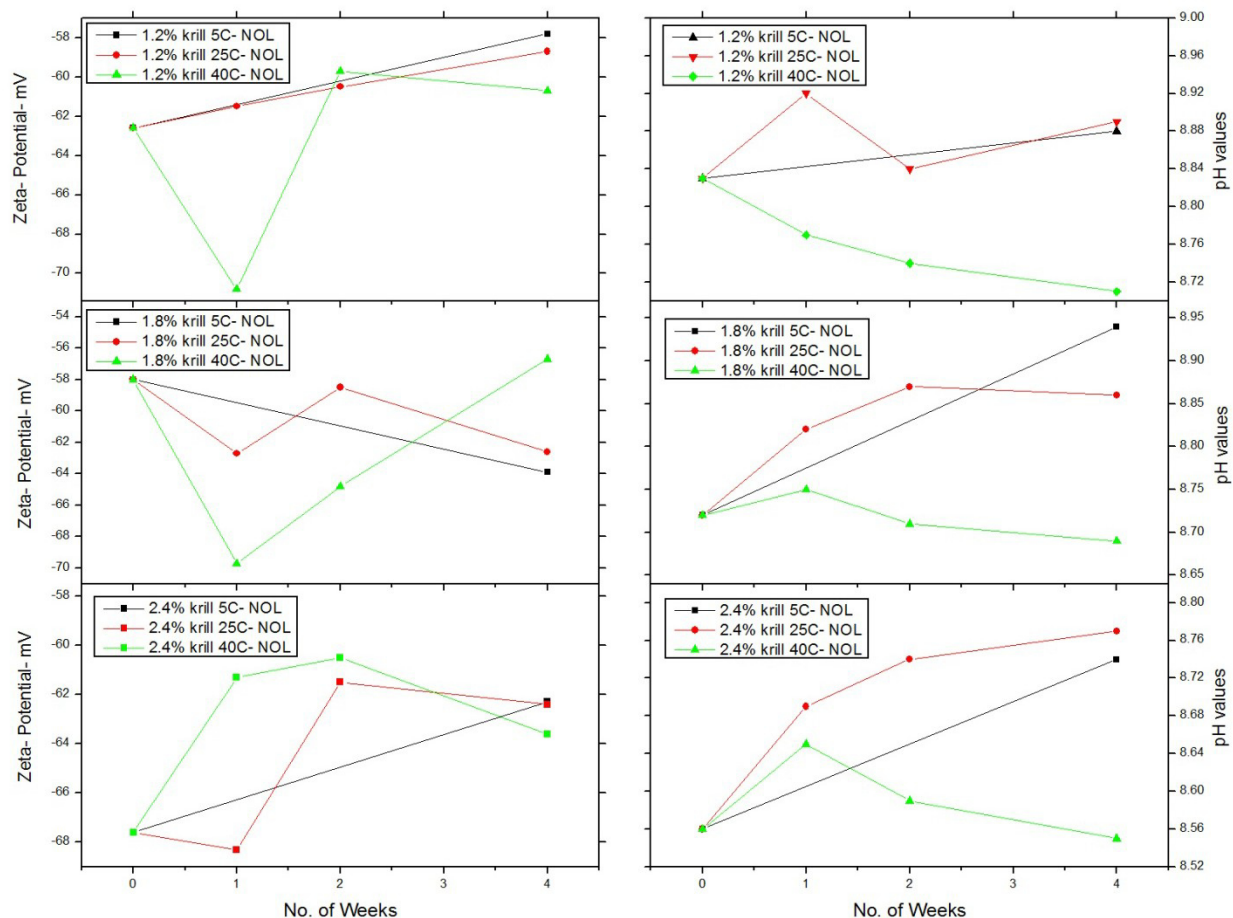


Figure 4.14: Variation of ζ - potential and pH values for 10% fish oil and different FKKO42 emulsifier formulations without sodium oleate addition along time at different storage temperatures.

In Figure 4.14, the evolution of pH values at 5 and 25 °C can be clearly observed, indicating an increasing trend of these values with respect to time for all formulations while a decreasing fashion was recorded for higher storage temperature (at 40 °C). For ζ - potential values, a mixed trend of both increasing and decreasing fashion was recorded at higher temperatures (25 and 40 °C). Briefly, at 40 °C storage temperature, ζ - potential (absolute) values were decreased with a magnitude of 3-4 mV from the corresponding fresh samples for all formulations after 4 weeks while 25 °C samples stored during 4 weeks, the ζ - values (absolute) were decreased for 1.2 and 2.4% FKKO42 emulsions while an increase was recorded for 1.8% formulations.

At 5 °C and 1.8% FKKO42 emulsions, the ζ - potential trend was opposite as compared to 1.2 and 2.4% FKKO42 formulation. The obvious reason in decreasing of this value (absolute) for 1.2

and 2.4% formulations, as discussed already, was suppression of hydrolysis product formation at lower temperature. While for 1.8% emulsifier oil, the increase in ζ - potential absolute value recorded was higher due to higher pH values of the same emulsions (Figure 4.14). This was obvious due to dependency of ζ - potential on pH principally. In this way, observing the corresponding pH value of the same emulsion at 5 °C, (measured 9 units instead of 8.7 for fresh samples) the ζ - potential values were measured higher too. So, there might be an inhibition of hydrolysis at lower temperature for 1.8% FKKO42 emulsions too, but the influence of increase in pH value gave an exaggerated value, experimentally. The trend of 1.2% formulation was identical to 2.4% formulation due to the fact that the naked oil particles, reported for this emulsion, might have influenced the values, due to their charging ability as commented by Driscoll, et. al., (2001) and Werling, et. al., (2008). Also, the change in value is not drastic and was in experimental error range (only a 2-3 mV difference). For 25 °C sample, it basically followed the same trend.

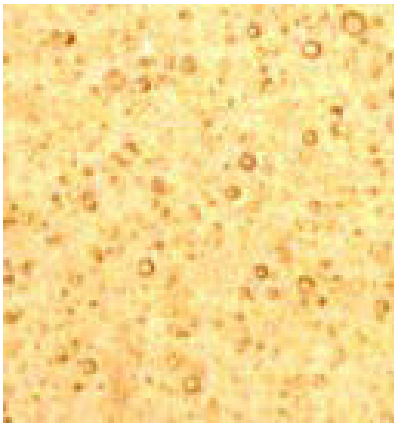
Density values were unchanged from 0.9961 gm/cm³ for 1.2 and 1.8% FKKO42 emulsions measured from freshly prepared samples till 4 weeks of storage time at different temperatures, while 0.9962 gm/cm³ was measured during storage time for 2.4% emulsions. Table 4.5 contains values of PFAT5 and osmolality measured for fresh samples at different FKKO42 additions.

Table 4.5: Osmolality and PFAT5 values of 10% fish oil with different FKKO42 percentages for fresh samples.

Fresh samples (Avg values)	Emulsifier oil Percentage		
	1.2	1.8	2.4
	PFAT5		
	0.041	0.022	0.0192
	Osmolality (mOsm/kg)		
	432	410	392

Micrographs of 10% fish oil without sodium oleate addition at various FKKO42 percentages and different storage time and temperatures are presented in Table 4.6. Visible emulsion drops were observed in images of 1.2% FKKO42 formulations at any temperature during corresponding storage time while fine droplets were observed for 1.8% FKKO42 and for the formulations concerning 2.4% emulsifier oil at any time and storage temperature. It was obvious that with a particle size range less than 170 nm, the magnification of 50x could not be enough to obtain a good micrographic image. The micrographs of selected formulations are presented in Table 4.6. Images were cropped and compressed with the help of Microsoft® picture manager tool and were auto corrected for appropriate size results as indicated in section 2.

Table 4.6: Microstructure of 10% fish oil with different FKKO42 emulsifier oil formulations freshly prepared and during different storage time and temperature conditions.

1.2% FKKO42 with 10% fish oil- Fresh sample	2.4% FKKO42 with 10% fish oil- Fresh sample
	
1.8% FKKO42 with 10% fish oil- 5 °C- 4 weeks	2.4 % FKKO42 with 10% fish oil- at 40 °C- 4 weeks
	

4.2.2 Formulations having 5% refined fish oil + 5% medium chain triglycerides (MCT) with and without sodium oleate.

In this section, formulations prepared with 5% refined fish oil and 5% MCT will be discussed along with different percentages of novel marine origin emulsifier i.e. FKKO42. Formulations may or may not comprise sodium oleate as was dealt previously in this study.

There were, however, no much difference between the two formulations and in this way, the stability study presented here in this thesis was on the basis of non oleate added emulsions.

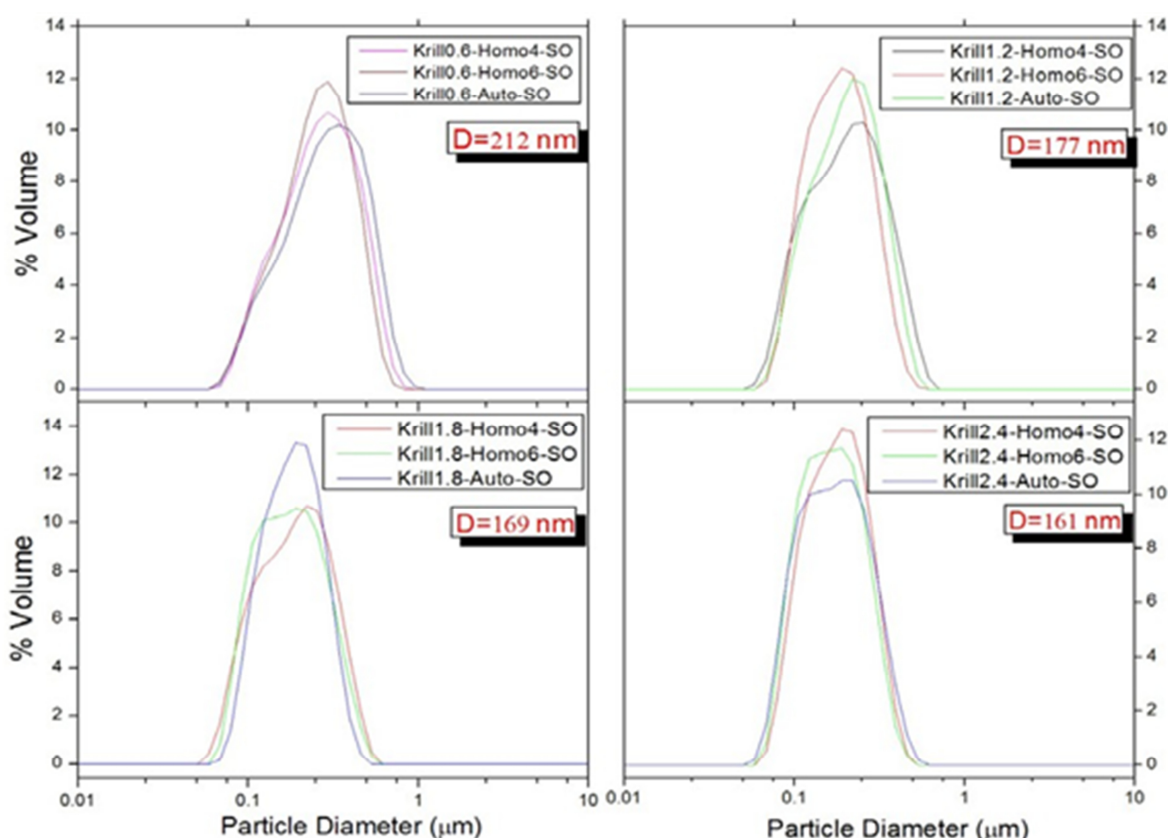


Figure 4.15: PSD curves of sodium oleate added 5% fish oil + 5% MCT formulations with different FKKO42 percentages (legend SO is sodium oleate and average particle size D, in nm, is written for autoclave treated samples only).

Particle size distribution curves obtained from the oleate added formulations at different FKKO42 percentage is presented in Figure 4.15. The emulsions with 0.6% emulsifier oil gave over 200 nm particle size range while all other formulations fell within 160-180 nm particle size range. Repetition was made for all these formulations without sodium oleate addition except for 0.6% FKKO42 emulsifier due to its higher particle size range and instability due to visible oil particles. Out of these formulations, 2.4% FKKO42 emulsions gave the minimum PSD range i.e. 160 nm

Figure 4.16 represents the particle size of non oleate added emulsions with 1.2 and 1.8% FKFO42 emulsifier oil formulations containing fish oil and MCT (5%+5%). There was no polydispersity found in 1.2% formulations as can be seen in Figure 4.16 and particle size for that formulation fell in a 190 nm range. The PSD range was similar to the formulations observed for other 10% oil loadings emulsions i.e. soy oil and/or fish oil. The emulsions were seemed stable for the respective FKFO42 emulsifier oil percentage. Micrographs of these formulations, presented at the end of this section, revealed more about the fact of smallest PSD for 10% oil loadings. Although particles were seen in images taken at 50x magnification, yet the distribution was very fine. In this way, not only 1.8% and 2.4% formulations are recommendable for this type of oil loadings, it might be considered that 1.2% FKFO42 emulsifier oil formulations were also appropriate.

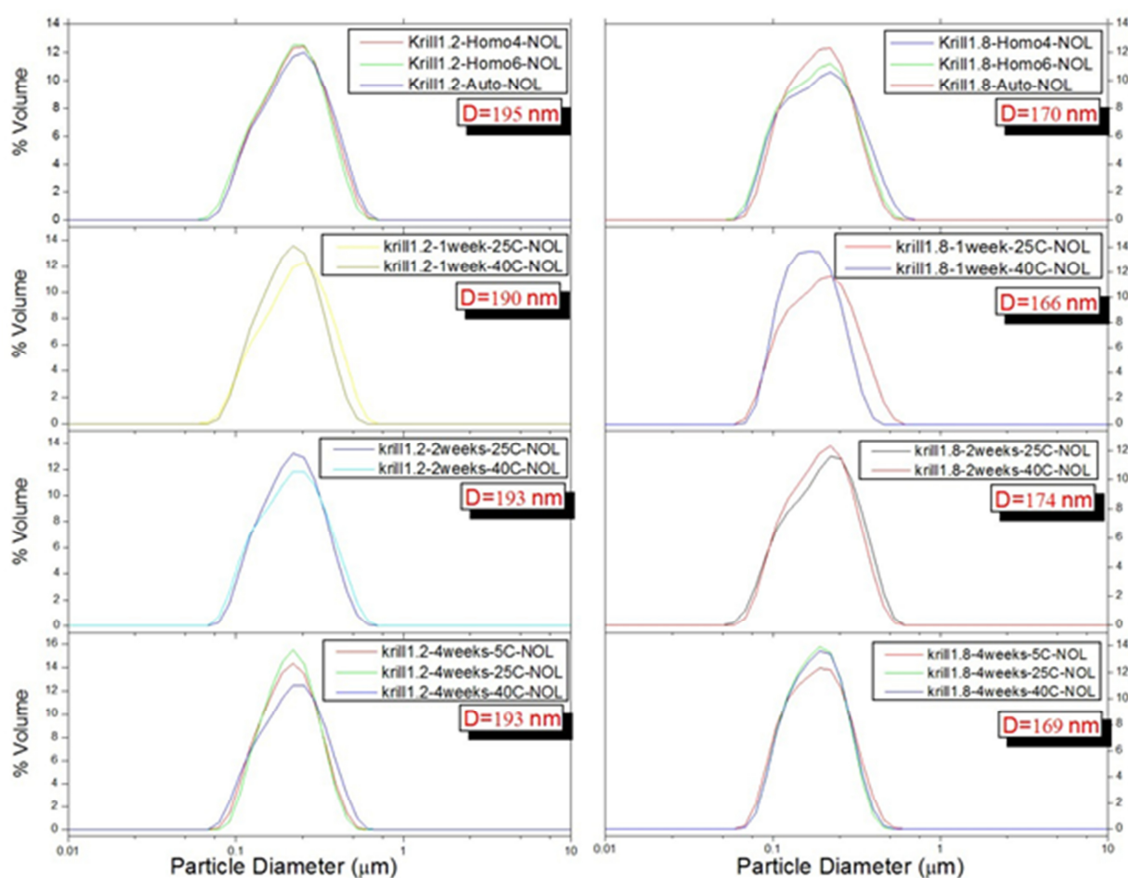


Figure 4.16: PSD curves of 5% fish oil + 5% MCT with 1.2 and 1.8 FKFO42 formulations without sodium oleate addition along time during different storage temperatures (legend NOL is no sodium oleate and average particle size D, in nm, is written for smallest sized samples only).

Figure 17 shows the PSD curves for 2.4% FKFO42 formulations along with mean particle sizes of 160 nm. 2.4% emulsifier oil was always found appropriate in terms of stability, PSD and Zeta potential during this study for approximately all formulations containing 10 or 20% oil loadings independent of the oil type.

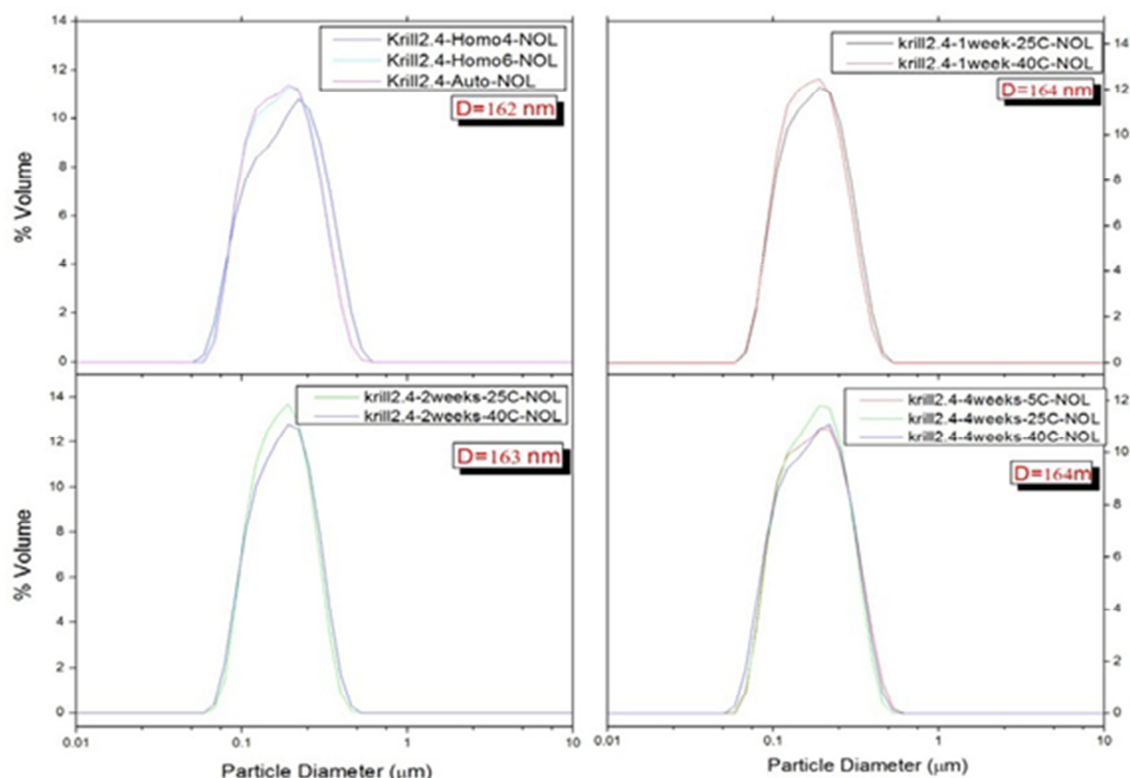


Figure 4.17: PSD curves of 5% fish oil + 5% MCT and 2.4% FKKO42 formulations without sodium oleate addition along time during different storage temperatures (legend NOL is no sodium oleate and average particle size D, in nm, is written for smallest sized samples only).

As far as ζ - potential of the oleate added formulations is concerned, these values fell in range from as low as -56 mV for 2.4% FKKO42 emulsifier oil while it was observed as high as -67 mV for 1.2% formulations. For ζ - potential values of formulations without sodium oleate addition, however, a complete set of measurements at each stability step (during storage time at different temperature) was made and presented here in this section. Figure 4.18 is a collective indication of ζ - potential and pH values of all the formulations under consideration with 5% fish oil + 5% MCT having different FKKO42 emulsifier oil percentages.

In Figure 4.18 for pH values, it is quite evident that the pH decreased at higher temperatures for all formulations while at 5 °C and 2.4% FKKO42 emulsifier oil, it was unchanged as compared to an increasing trend for the other two formulations (1.2 and 1.8% FKKO42) at the same temperature.

As far as ζ - potential values are concerned in Figure 4.18, at 25 °C, the values fell in the same range after a study period of 4 weeks while at 40 °C and 2.4% FKKO42, the formulations were also found in the same ζ - potential range after 4 weeks but 1.2 and 1.8% FKKO42 emulsifier oil formulations, the ζ - potential (absolute) values were decreased as compared to their initial values measured for fresh samples.

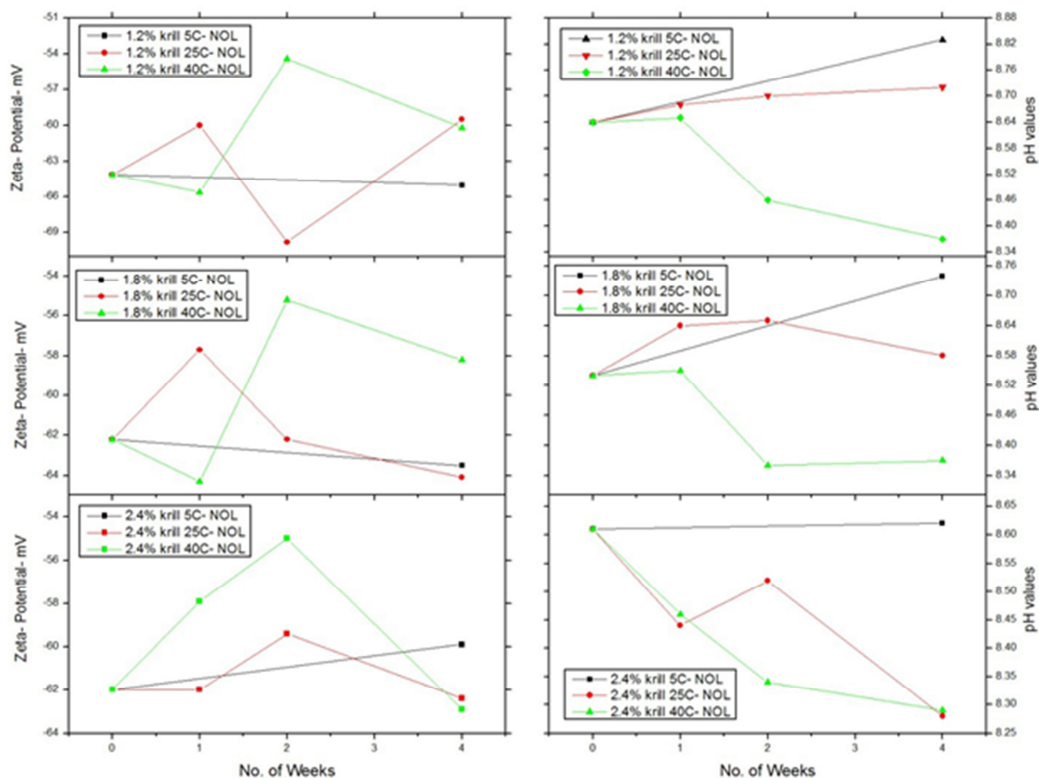


Figure 4.18: Variation of ζ - potential and pH values for 5% fish oil + 5% MCT and different FKKO42 emulsifier oil formulations without sodium oleate addition along time at different storage temperatures.

At storage temperature of 5 °C, this trend was different than was observed for other formulations studied in previous sections 4.1.1 and 4.2.1 for 10% soy and fish oil formulations respectively. There was no big difference observed for 1.2 and 1.8% FKKO42 formulations and values fluctuate around -62 to -64 mV for both formulations after 4 weeks of study. The same argument holds true for 2.4% formulations too where the values fell between -62 to -60 mV indicating not an observable change in the ζ - potential values along with the storage time and temperature.

Density values were unchanged from 0.9969/0.997 gm/cm³ for 1.2 and 1.8% FKKO42 formulations measured from freshly prepared samples till 4 weeks of storage time at different temperatures while 0.997/0.9971 gm/cm³ was measured during storage time for 2.4% FKKO42 formulations. PFAT5 values measured for fresh samples are given in Table 4.7.

Table 4.8 contains information of micrographs of above mentioned formulations during time at different temperatures. Again, particles of 1.2% FKKO42 formulations were visible under microscope while for 1.8% FKKO42 oil, though particles were visible, yet very small. For 2.4% FKKO42 emulsifier oil formulations, nothing was very clear at the magnification of 50x used in this study. The images were processed with the help of Microsoft picture manager tool to compress and crop for the appropriate size.

Table 4.7: PFAT5 and osmolality values for 5% fish oil + 5% MCT fresh samples at different percentages of FKKO42 emulsifier oil.

Fresh samples (Avg values)	Emulsifier oil Percentage		
	1.2	1.8	2.4
	PFAT5		
	0.033	0.020	0.0182
	Osmolality (mOsm/kg)		
	396	411	386

Table 4.8: Microstructure of selected 5% fish oil + 5% MCT without sodium oleate and different FKKO42 emulsifier oil formulations at different storage time and temperatures.

1.2% FKKO42 with 5% fish oil + 5% MCT - Fresh sample	2.4% FKKO42 with 5% fish oil + 5% MCT - Fresh sample
	
1.8% FKKO42 with 5% fish oil + 5% MCT - 5 °C- 4 weeks	2.4 % FKKO42 with 5% fish oil + 5% MCT - at 40 °C- 4 weeks
	

As compared to 10% fish oil alone, a mixture of fish oil with MCT (5% each) gave a better set of formulations with different FKKO42 formulations. It was further observed in the stability vials stored at 25 °C for 4 weeks storage time, that there were no naked oil drops and/or characteristic emulsifier oil layer. Especially for formulations at 2.4% FKKO42 emulsifier oil, it represented the best set of emulsions throughout the study. This formulation is highly recommended for practical purposes and for all the FKKO42 percentages i.e. 1.2/1.8 and 2.4%. Another recommendation for these formulations is a preferred preparation without sodium oleate addition as indicated by the results presented here in this section.

4.2.3 Formulations having 10% omegaven with and without sodium oleate

Omegaven is concentrated fish oil containing EPA as 20.5 g/100g sample and DHA as 17.3 g/100g sample according to certificate of analysis given by the supplier (Fresenius- Kabi GmbH) while a total of 37.8g omega-3 acids per 100g sample were found in this concentrate. Apart from its nutrient value in the parenteral emulsions, here the discussion will be made with reference to the manufacturing aspects of this formulation. FKKO42 emulsifier oil with different percentages i.e. 0.6/1.2/1.8 and 2.4% was used along with the addition of sodium oleate. While same formulations were repeated without sodium oleate addition and results are presented here in this section.

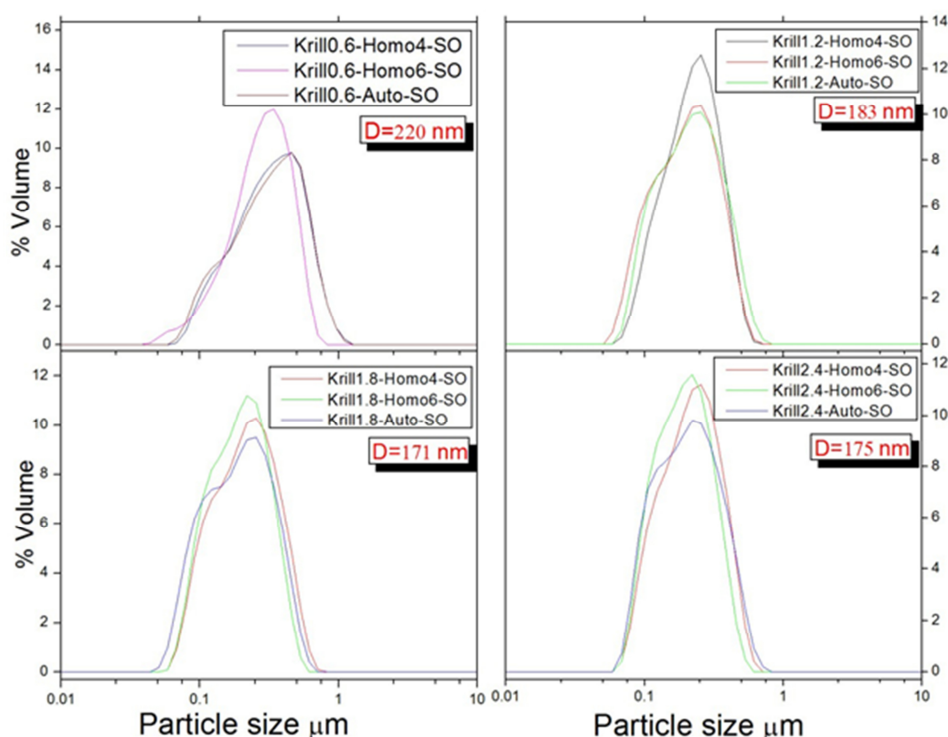


Figure 4.19: PSD curves of sodium oleate added 10% omegaven formulations with different FKKO42 emulsifier oil (legend SO is sodium oleate and average particle size D, in nm, is written for autoclave treated samples only).

Firstly, results will be presented for the sodium oleate added formulations with respective PSD curves for FKKO42 emulsifier oil formulations and 10% omegaven are presented in Figure 4.19. The lowest mean particle size was obtained in the range of 170 nm for 1.8 and 2.4% emulsifier oil while more than 200 nm particle size was obtained for 0.6% FKKO42 formulations and a merely 200nm mean particle size was obtained for 1.2% formulations.

Formulations with 0.6% FKKO42 seemed unstable and inappropriate quantity of emulsifier was the possible reason behind the instability. No repetition was made without sodium oleate addition with this percentage of FKKO42 emulsifier oil.

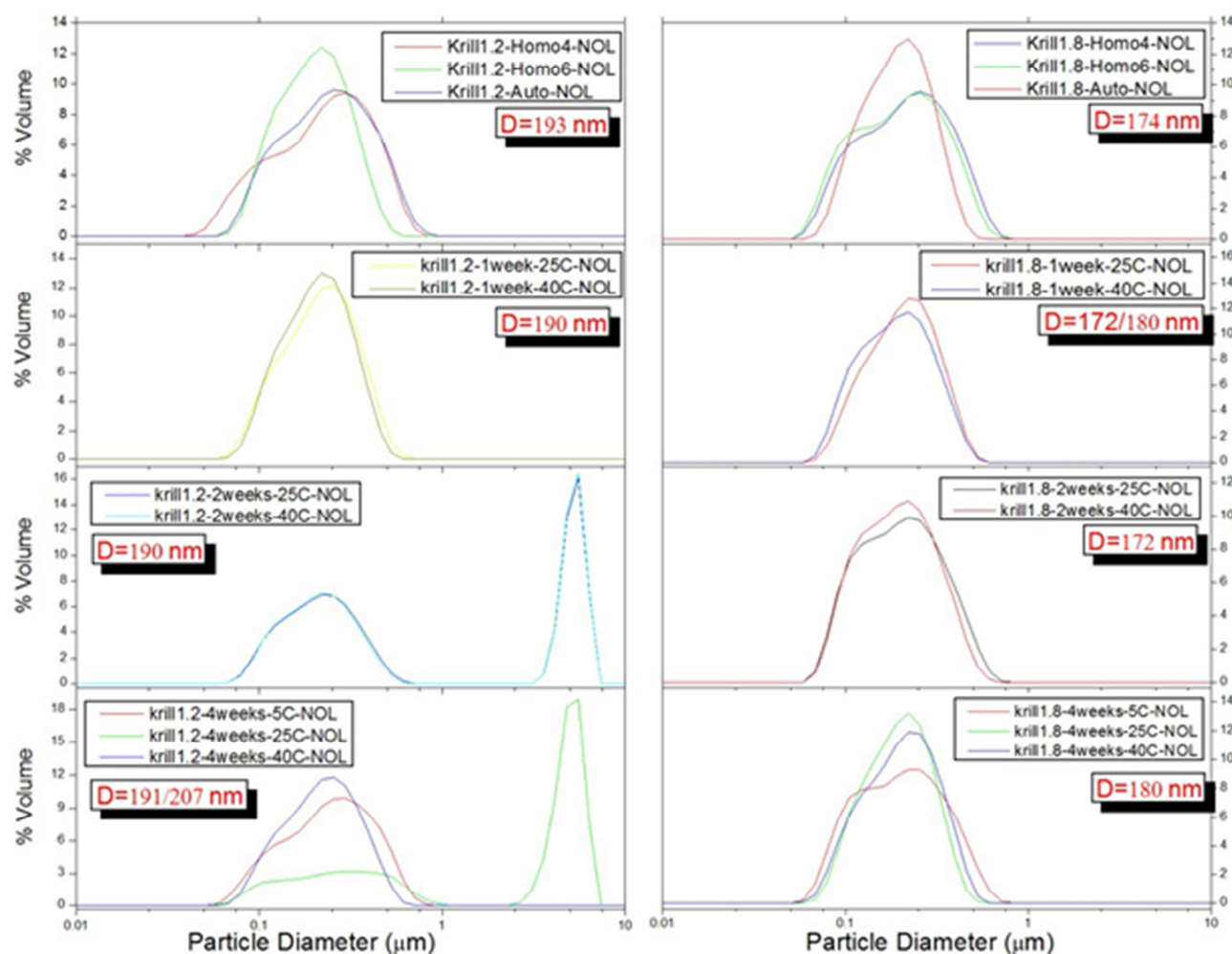


Figure 4.20: PSD curves of 10% omegaven with 1.2 and 1.8% FKKO42 emulsifier oil formulations without sodium oleate addition along time during different storage temperatures (legend NOL is no sodium oleate and average particle size D, in nm, is written for smallest sized samples only).

The formulations were practically stable after 4 weeks at different temperatures for all concentration of FKKO42 except for 0.6%. The minimum PSD was obtained for 2.4% FKKO42

emulsifier oil and is presented in Figure 4.21 indicating a 160 nm particle size and even without sodium oleate addition.

The PSD of 1.2 and 1.8% FKKO42 emulsifier oil without sodium oleate addition is presented in Figure 4.20 whereby indicating a polydispersity along time in the formulations at 1.2% emulsifier oil concentration having 200 nm particle size after 4 weeks stability period. Similarly at 1.8% FKKO42 concentration, the emulsions were stable and 170-180 nm particle size was obtained after 4 weeks stability period.

In this way 1.8 and 2.4% FKKO42 formulations were excellent candidates for their practical/industrial use in reference to their characteristic stability.

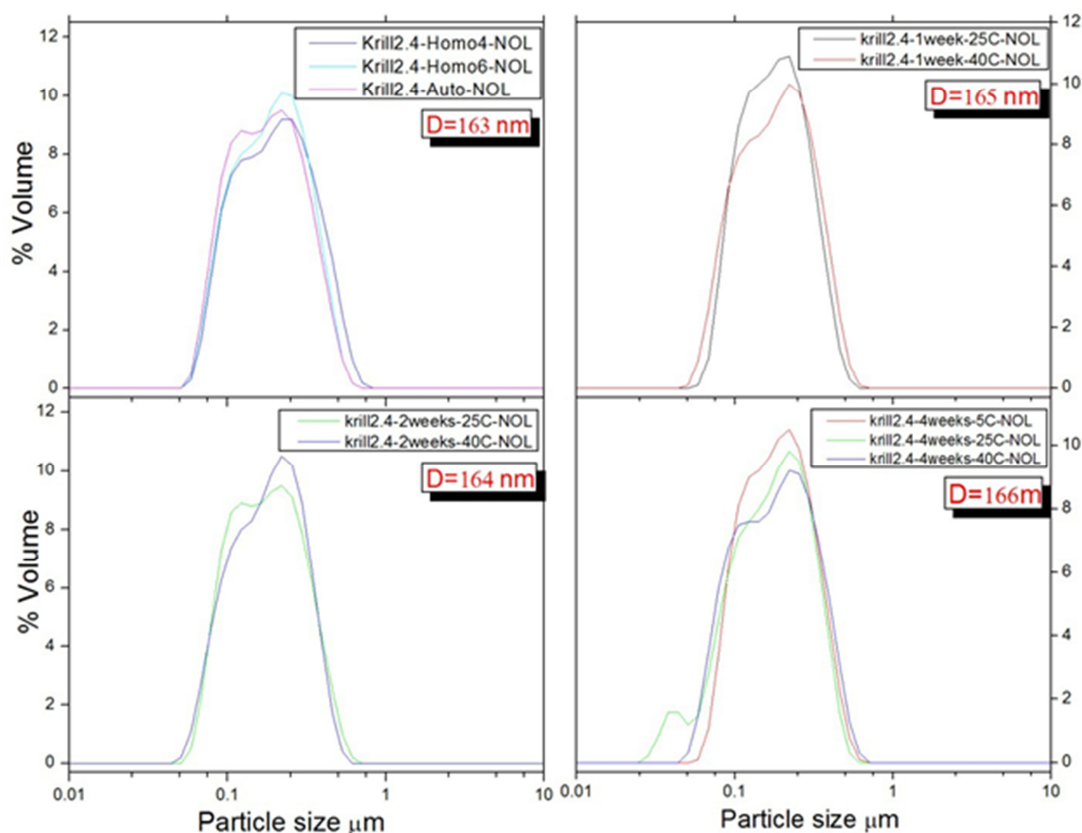


Figure 4.21: PSD curves of 10% omegaven and 2.4% FKKO42 formulations without sodium oleate addition along time during different storage temperatures (legend NOL is no sodium oleate and average particle size D, in nm, is written for smallest sized samples only).

In measuring ζ - potential values for the emulsions with sodium oleate addition, the values obtained were varied between as low as -51 mV for 0.6% formulation while towards higher end; these values were measured -63 mV for 2.4% FKKO42 emulsions. The variation of ζ - potential values along time at different storage temperatures are presented in Figure 4.22.

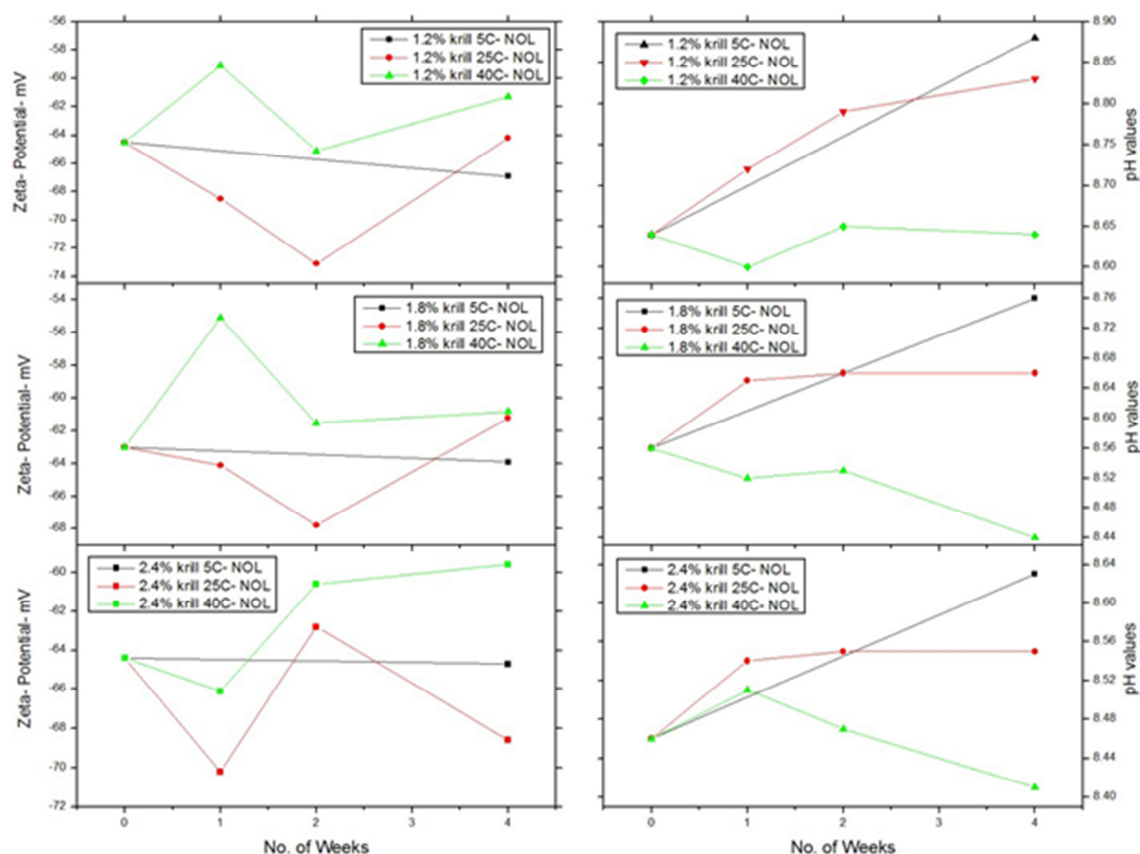


Figure 4.22: Variation of ζ - potential and pH values for 10% omegaven and different FKKO42 formulations without sodium oleate addition along time at different storage temperatures.

According to the pH graph depicted in Figure 4.22, it might be clearly seen that the pH values of all formulations at 5 °C were increased. The highest change was observed for the 1.2% formulations while 1.8 and 2.4% formulations, pH values increased similar to that of 1.2% formulations at that temperature, i.e. 5 °C. Formulations stored at higher temperature decreased their values during time while at room temperature storage, all formulations exhibited same trend.

As far as ζ - potential values of omegaven 10% formulations were concerned, the higher temperature storage of all formulations yielded similar trend i.e. either they were unchanged or a small decrease (in terms of absolute values). At 5 °C the ζ - potential values for all formulations did not change either or the values were within experimental error range i.e. $\pm 2 - 3$ mV. This trend at 5 °C was marked contrary to the other emulsions studied in this section with other types of oils in the formulations, for instance (soy oil and fish oil or its mixture with MCT etc).

The possible reason of not changing the ζ - potential values of the formulations under consideration might be related to the behavior of the oil added. For example, in 1.2% emulsions, even if the pH value was recorded higher, the ζ - potential value was measured lower during time

as compared to its measurement for freshly prepared emulsion. There was an evidence of naked oil particles at the top on the same formulations too, though not indicating any change in the ζ -potential values. PFAT5 values also indicated a higher percentage for 1.2% FKKO42 emulsions reinforcing the fact for naked oil drops, yet in tolerable range of USP <729> and is indicated in Table 4.9.

Density value was measured 0.9974 gm/cm^3 for 1.2 and 1.8% FKKO42 formulations from freshly prepared samples while a 0.9975 gm/cm^3 was measured for 2.4% formulations. Similarly, the osmolality values are also presented in Table 4.9.

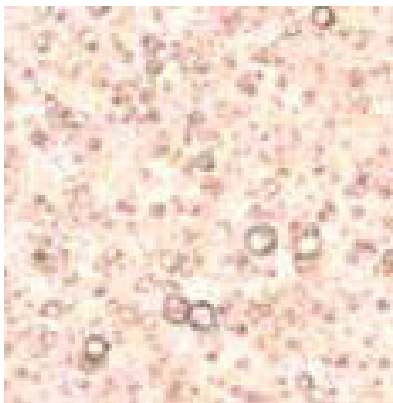
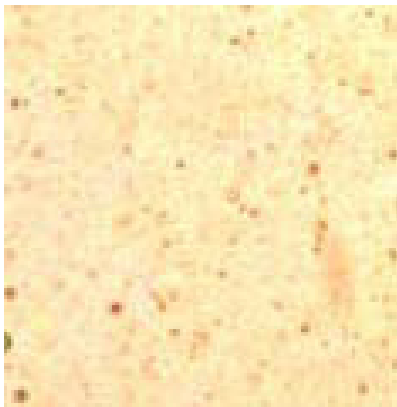
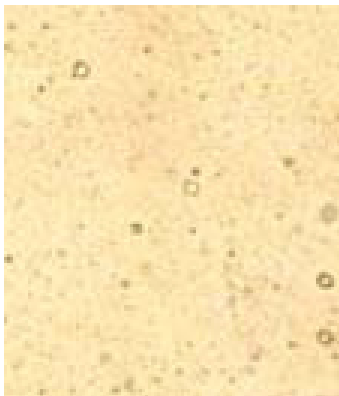
Table 4.9: PFAT5 and Osmolality values of freshly prepared emulsions containing FKKO42 emulsifier oil and 10% omegaven.

Fresh samples (Avg values)	Emulsifier oil Percentage		
	1.2	1.8	2.4
	PFAT5		
	0.0291	0.0202	0.0196
	Osmolality (mOsm/kg)		
	452	435	397

Micrographs were obtained during stability study at different storage time and temperature. Table 4.10 is a self-indicative of few micrographic images taken during this study at different stages with different FKKO42 emulsifier oil formulations. Micrographs were cropped and compressed to size in the table using Microsoft picture manager tool.

Omegaven contains more than 37% of combined omega-3 acids, which, in reality is a concentration amount in excess. Like fish oils, concentrated fish oil has an even higher risk of deterioration due to oxidation because of a higher content of EPA and DHA which are prone to oxidation. Emulsifier for formulations containing omegaven, in this way, became a very important parameter in order to protect the oil content or otherwise a deteriorated product may result. FKKO42 emulsifier oil with 1.8% and 2.4% concentration resulted as a suitable emulsifier concentration for the studies performed during this period of time, i.e. 4 weeks at different temperatures. There was however, a very small characteristic emulsifier layer that was reported earlier in other types of formulations too, but very small evidence was found for 1.8 and 2.4% FKKO42 formulations. While naked oil drops were concerned quite indifferently and were marked in 1.2% FKKO42 emulsions. Due to observable free oil drops in FKKO42 with 1.2% concentration, emulsions with 1.8 and 2.4% FKKO42 emulsifier oil were only considered appropriate for the subsequent study.

Table 4.10: Microstructure of 10% omegaven and different concentration of FKKO42 formulations without sodium oleate at different storage time and temperatures.

1.2% FKKO42 with 10% omegaven- Fresh sample	2.4% FKKO42 with 10% omegaven- Fresh sample
	
1.8% FKKO42 with 10% omegaven- 5 °C- 4 weeks	2.4 % FKKO42 with 10% omegaven- at 40 °C- 4 weeks
	

4.3 SMOF (Soy oil + MCT + Olive oil + Fish oil) formulations

The discussion regarding using alternative oils other than soy oil in o/w IV emulsions for prolonged therapeutic has already been made in the 2nd part of this thesis. In this sense, fish oil (refined and concentrated) was also used in 2nd part, however, in this part; SMOF was also used in the formulations of o/w emulsions as commented earlier in part II.

In relation to SMOF oil mixtures, an introductory note has already been made in the part II, about the significance of this oil mixture as compared to soy oil emulsions. SMOF is preferred oil phase in commercial emulsions nowadays in replacement to soy oil due to its advantages over the long term therapy. the clinical role of soybean oil based lipid emulsions compared to alternative oils lipid emulsions (Fish, olive, omegaven and mixture of oils known as SMOF) and concluded that alternative oil based lipid emulsions have fewer pro-inflammatory effects and have stronger antioxidant effects so alternate oil emulsions are superior to soybean oil lipid emulsions used commercially, even now as reviewed by Vanek et. al. (2012). On a similar note, Wanten, (2015) concluded the same results specifically for fish oil emulsions as compared to the soy oil based lipid emulsions due to the fact that fish oil is rich in PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA modulate inflammatory response to improve immune-mediated conditions as suggested by Kim et. al. (1983).

In this way, this study contained the use of SMOF as well, for the first time with new marine based emulsifier systems i.e. FKKO42, in the o/w emulsions.

Formulations with a mixture of different types of oils were prepared in this study as indicated in materials section (section 2). Results obtained by emulsifying 0.6/1.2/1.8 and 2.4% FKKO42 emulsifier oil using sodium oleate and without its presence are presented. It is out of the scope of this study to comment on the question why to formulate the mixture of oils within one confined formulation whereby the nutrient capacity of these oils and their use in parenteral emulsions have been discussed by many authors (Mertes et al., 2006; Klek et al., 2013; Tomsits et al., 2010 and Goulet et al., 2010). Shortly, a mixture of soy oil, medium chain triglycerides, olive oil and fish oil was formulated with the help of emulsifier oil (FKKO42) with different percentages as indicated above. The total nutrient percent was counted as 20% in SMOF having 6% soy, 6% MCT, 5% olive and 3% fish oil as indicated in materials section (section 2).

Firstly, sodium oleate formulations will be discussed in this section and a repeated preparation of emulsions without its addition will be discussed thereafter.

Figure 4.23 indicates the PSD curves of different percentages of FKKO42 emulsions formulated with SMOF in presence of sodium oleate.

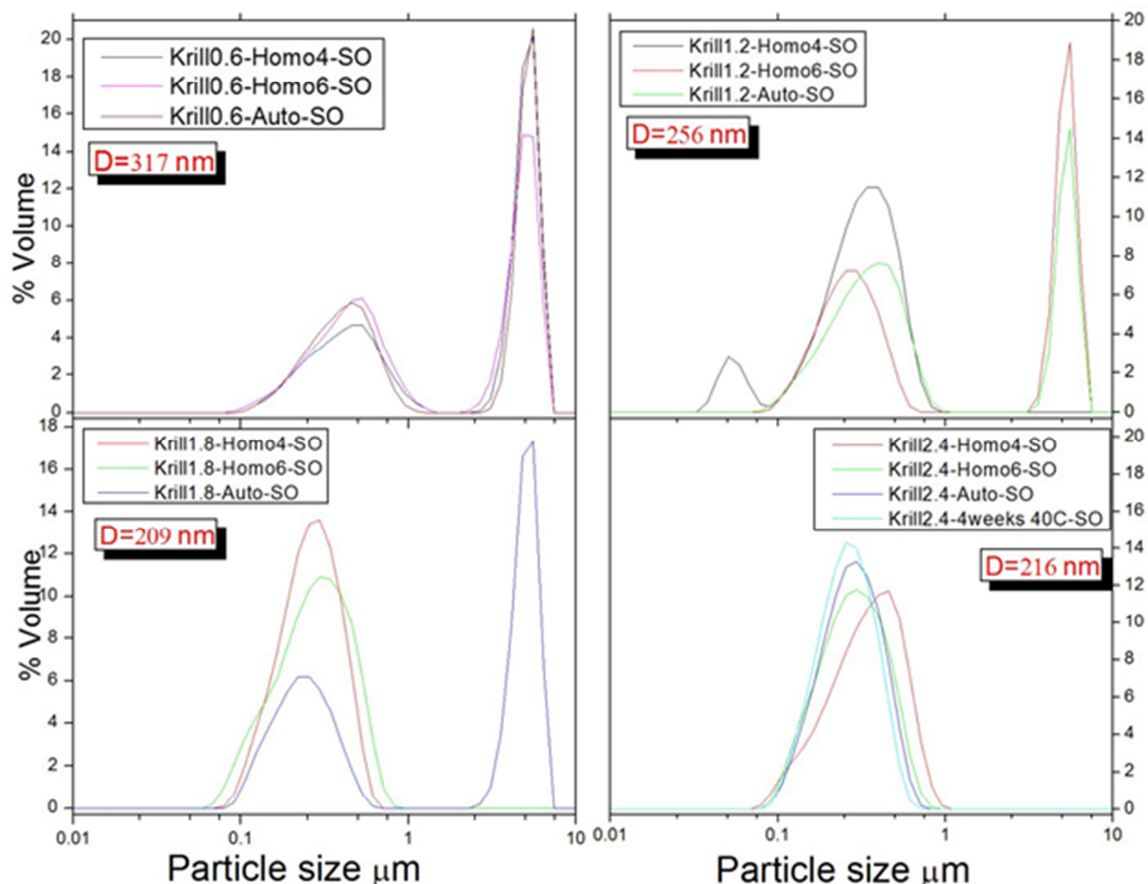


Figure 4.23: PSD curves of sodium oleate added 20% SMOF formulations with different FKKO42 emulsifier oil (legend SO is sodium oleate and average particle size D, in nm, is written for autoclave treated samples only).

Polydispersity was observed in all the formulations in Figure 4.23 with an exception of 2.4% emulsions where throughout the stability period studied, a PSD of 200 nm particle size was measured. Similarly, for 1.8% formulations, the particle size was observed around 209 nm with a slight polydispersity whereas for the formulations with 0.6 and 1.2% FKKO42, the particle size was observed between 250 and over 300 nm indicating higher polydispersity. Also, naked/free oil drops were observed for these two formulations (0.6 & 1.2%) apart from emulsifier oil layer. In this way, 0.6 and 1.2% formulations were not prepared without sodium oleate. At higher loading, such as 20% oil, it was quite obvious that a higher quantity of emulsifier must be needed to emulsify the water/oil interphase.

Figure 4.24 consists of PSD curves of 2.4% FKKO42 formulations studied versus time (during 6 weeks)

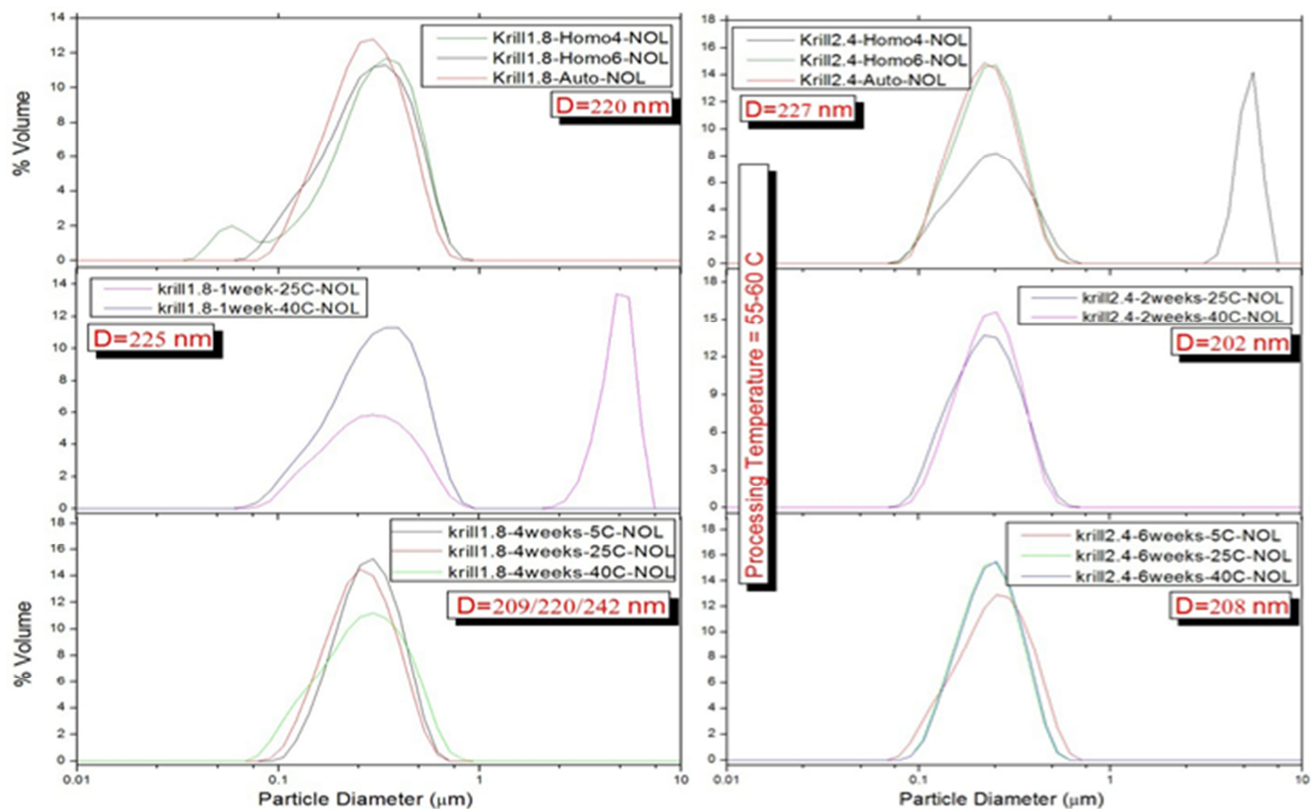


Figure 4.24: PSD curves of 20% SMOF formulations with 1.8 and 2.4% FKFO42 without sodium oleate addition (legend NOL is no sodium oleate and average particle size D , in nm, is written for autoclave treated samples only).

Figure 4.24 indicates a 200 to 240 nm particle size for all the formulations prepared without sodium oleate, in 1.8 and 2.4% FKFO42 emulsifier oil. In all cases, there were oil drops confused to be characteristic emulsifier oil layer at the top of the emulsions. Figure 4.25 indicates the evidence of this layer around the circumference of the vials. For 2.4% FKFO42 emulsions, however, it was even more enhanced and can be seen clearly in Figure 4.25. Similarly, for 1.8% emulsions, broader PSD curve was obtained.



Figure 4.25: Image showing closed vials of 1.8 and 2.4% FKFO42 emulsifier oil with 20% SMOF formulations without sodium oleate addition after 4 and 6 weeks of stability for 1.8 and 2.4% FKFO42 emulsions at 25 °C indicating free oil drops at top.

Measurement of ζ - potential values for sodium oleate added formulations have absolute values in the range of -69 mV for 2.4% FKFO42 emulsions while the value for 0.6% FKFO42 was calculated to be -57 mV. For 0.6% and 1.2% FKFO42 emulsifier oil formulations with sodium oleate addition, visible oil drops at the top were observed and were marked unstable.

Figure 4.26 below indicates the change in ζ - potential values of FKFO42 emulsions with different percentages stored at various temperatures along time. Figure 4.26 also refers changes in pH values along time at different temperatures with FKFO42 emulsifier.

The pH values were increased during storage at lower (5 °C) and at room temperature for all formulations while this value decreased at higher temperature. Similarly, ζ - Potential (absolute) values at 25 and 40 °C were decreased along time for all formulations while it showed a different response for 2.4% FKFO42 emulsions prepared at room temperature and indicated no change when observed at 5 °C along time. The other two formulations (1.8% and 2.4% FKFO42 emulsions prepared with normal protocol i.e. 55-60 °C) indicated an increase in their ζ - potential absolute values along time at that temperature. The presence of free oil or aggregation of emulsifier layer, as defined previously above, might be the cause of the change in values for these two formulations.

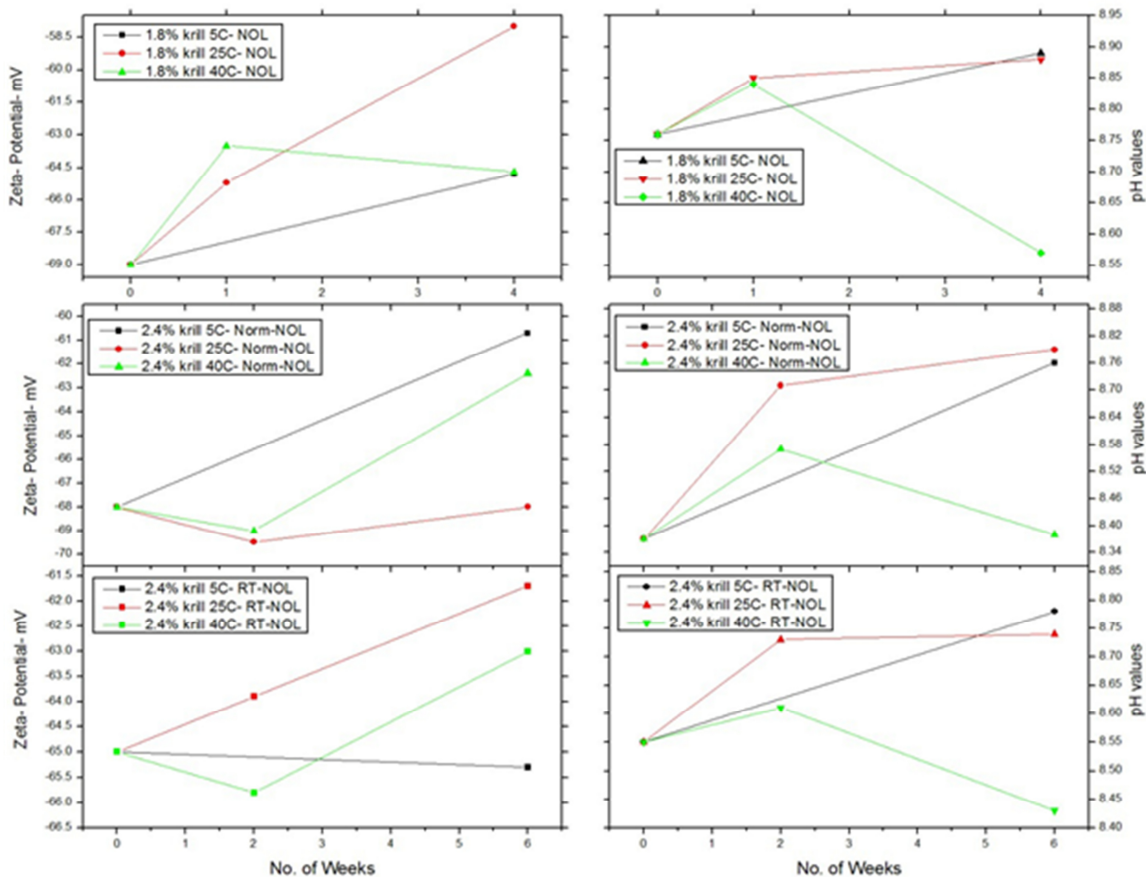


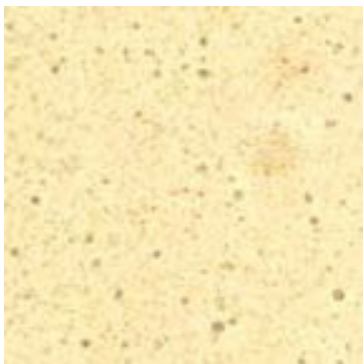
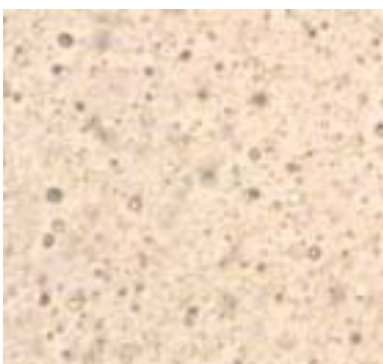
Figure 4.26: Variation of ζ - potential and pH values for SMOF with 1.8 and 2.4% FKFO42 emulsifier oil formulations without sodium oleate addition along time at different storage temperatures.

Density values of FKFO42 emulsifier oil with sodium oleate additions were measured for 1.8% formulations as 0.9878 g/cm^3 while that of 2.4% formulations were 0.9884 g/cm^3 . Due to free oil droplets at the top of 1.8 and 2.4% FKFO42 emulsions, PFAT5 values were not calculated for these formulations and similarly osmolality was also not considered for these emulsions. In fact, PFAT5 values were out of range due to oil presence (i.e. PFAT5 of 2.4% formulation was 0.0687).

Microscopic characterization in relation to the SMOF formulations was also carried out and Table 4.11 gave a clearer picture of the evolution of microstructure during storage at different temperatures along storage time. As explained in previous sections, the originally taken micrographs were stored and achieved as such while for size limitations and appropriate visibility, Microsoft picture manager tool was used to crop and auto correct the images.

Micrographs of prepared formulations with 1.8 or 2.4% FKFO42 emulsifier oil, as presented in Table 4.11, indicates visible emulsion drops.

Table 4.11: Microstructure of freshly prepared SMOF with 1.8% and 2.4% FKKO42 formulations without sodium oleate addition and at different storage time and temperature.

1.8% FKKO42 with SMOF- Fresh sample	2.4% FKKO42 with SMOF- Fresh sample
	
1.8% FKKO42 with SMOF- 5 °C- 4 weeks	2.4 % FKKO42 with SMOF- at 40 °C- 6 weeks
	

SMOF formulations are special formulations in terms of their oil loadings percent and also in their variety of oil mixture presence. In this way, SMOF emulsions are a very good candidate in pharmaceutical studies to have diverse applications in nutrition. Two different emulsifier oil formulations with 1.8 and 2.4% FKKO42 were characterized in this study to develop a stability correlation based on previous experience. Both formulations have an enhanced emulsifier layer at the top that also segregated during storage time and temperature and confused with oil like droplets as can be seen in Figure 4.25. Closed vials were observed under florescent light in order to distinguish between FKKO42 emulsifier oil layer and naked oil drops and help was taken from micrographic images and PSD curves as well.

Characterization results indicate that 2.4% FKKO42 emulsifier oil percentage is a better option comparing the two formulations (1.8 and 2.4% FKKO42). 1.8% FKKO42 emulsifier oil though seemed stable but its particle size was reported more polydisperse with a pronounced evidence of the dual emulsifier oil layer and naked oil particles at the top. 2.4% FKKO42 emulsions gave appropriate percentage of emulsifier and have an advantage of nutrients values higher in the form of marine emulsifier addition within an emulsion.

5. Conclusion

Based on discussions with two different products in this section, following are the conclusions drawn on the basis of results presented above in section 3 and 4 respectively.

5.1 New emulsifiers for Parenteral Emulsions: FKHEO30

After a brief discussion on FKHEO30, following are the conclusions derived from the results and discussion section.

- No formulation survived beyond 1.2% FKHEO30 emulsifier independent of the oil loading.
- No matter what FKHEO30 emulsifier percentage was, oil loadings more than 10% never survived any formulation independent of the oil type. For instance, 20% soy oil or SMOF were not possible to formulate with FKHEO30 emulsifier.
- Formulations with 10% oil loadings were possible to emulsify with FKHEO30 emulsifier using 0.6 and 0.9% concentrations. However, the PSD curves obtained were polydisperse yet overall values near 200 nm, indicating free oil drops with visual observation as well.
- Soy oil emulsions with 10% loadings, fish oil and Fish + MCT with 10% formulations showed characterization values in stable range but visual inspection and evidence from PFAT5 did not comply with the stability criteria.
- Formulations with omegaven containing 10% concentration were not stable with any FKHEO30 emulsifier percentage, though very good PSD range initially yet free oil drops presence.
- PEG 400 containing emulsions showed better characteristics values for PSD, ζ - potential and pH for instance for all characterized and formulated emulsions, however, the stability criteria was not met though.

- With 0.6 and 0.9% FKHEO30 emulsifier oil, the PFAT5 values were always higher and out of specs indicating free oil droplets in the formulations.
- The time period studied and the storage temperature considered for the formulations stability, FKHEO30 was not considered an appropriate emulsifier for commercial formulations.

5.2 New emulsifiers for Parenteral Emulsions: FKKO42

After a brief discussion on FKKO42, following are the conclusions derived from the results and discussion section.

- With FKKO42 emulsifier, only 0.6% emulsifier concentration was not appropriate for formulations to emulsify the o/w emulsions.
- Emulsifier concentrations of 1.8 and 2.4% were appropriate in most of the cases for emulsions formulations.
- Soy oil with 10 and 20% loadings and 1.8% or 2.4% FKKO42 emulsifier concentration were considered adequate for stability study at different temperatures.
- For Soy 30% loadings, studied FKKO42 concentrations were not enough for the emulsification of these formulations.
- Fish oil with 10% loadings, fish oil and MCT with 5% each oil loadings and omegaven 10% oil loadings, FKKO42 with 1.8% concentration was appropriate choice for stability study at various temperatures.
- SMOF with 2.4% FKKO42 might be considered a candidate for stability study but the characteristic layer of emulsifier confused free oil drops on the emulsion formulation and was evidenced with the help of PFAT5 evaluations too.
- FKKO42 with 1.8 and 2.4% concentrations might be considered an appropriate candidate for the commercial formulations studied with the oil loadings in this research work.

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