Faculty of Nursing, Pharmacy and Health Professions

Master Program in Industrial Pharmaceutical Technology

Soft gel capsule of Atorvastatin and Ezetimibe self-micro emulsifying drug delivery system

تحضير كبسولة هلامية لينة من مادتي الأتورفاستاتين والإزيتيميب في نظام توصيل الدواء نانوي و ذاتي الاستحلاب

By

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Dedication

This is dedicated to the only two persons who deserve it, my parents.

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List of abbreviations and symbols

Abbreviation	Description
BCS	Biopharmaceutical Classification System
Conc.	Concentration
Co-surf.	Co-surfactant
HMG-CoA	3 hydroxy-3-methylglutaryl coenzyme A
DLS	Dynamic light scattering
EM	Electron microscope
FDC	Franz diffusion cell
FTC	Freeze thaw cycle
GI	Gastrointestinal
HLB	Hydrophilic – lipophilic balance
HPLC	High performance liquid chromatography

ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
EZE	Ezetimibe
ATV	Atorvastatin
J_{ss}	Steady state flux
K	Partition coefficient
LOD	Limit of detection
LOQ	Limit of quantitation
Max.	Maximum
M.C.T	Medium chain triglycerides
ME	Microemulsion
HDL-C	High density lipoprotein – cholesterol
LDL-C	Low density lipoprotein – cholesterol
W/O	Water in Oil
O/W	Oil in water
P	Permeability coefficient
PEG 400	Poly ethylene glycol 400
RH40	Kolliphor® RH 40 (Polyoxyl 40 hydrogenated castor oil)
RI	Refractive index
RPM	Round per minute

RT	Room temperature
SD	Standard deviation
SEM	Scanning electron microscope
t	Flow time
T80	POE-20-sorbitan monooleate (Tween 80)
TEM	Transmission electron microscopy
TL	Lag time
USP	United States Pharmacopeia
UV	Ultra violet
WS	Working standard

Abstract

Atorvastatin and Ezetimibe are two drugs that have such a low oral bioavailability (14% and 35% respectively), and high presystemic clearance, and/or extensive first-pass metabolism, due to that both drugs come under the Biopharmaceutics Classification System (BCS) class II.

Among primary and secondary prevention treatments of cholesterol, 3 hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, inhibit cholesterol synthesis in the liver. Ezetimibe (EZE) also functions as a cholesterol absorption inhibitor.

However, integrating both drugs into one drug delivery system is very challenging due to their varying physicochemical properties.

This research comprises a self-micro-emulsifying drug delivery system encapsulated in a soft gel capsule containing Atorvastatin with Ezetimibe. This study's essential scope is to increase bioavailability using a soft gel self-micro emulsifying drug delivery system.

The study included pharmaceutical development, as well as physical and chemical characterization, stability assessments, and in vitro dissolution studies.

The ATV/EZE self-micro emulsifying system was formulated employing 10 mg of Ezetimibe, 40 mg of Atorvastatin, oleic acid or Triacetin as the oil phase, along with the surfactants Tween 80, Tween 20, or Kolliphor RH40 and co-surfactants PEG 400 or Propylene Glycol.

Among all the investigated formulations- using Pseudo-ternary phase diagrams- 45 were found to have a distinct clear monophasic configuration. Subsequently, five of these formulations (**ME#12, ME#22, ME#26, ME#31, ME#35)** successfully met the physical requirements concerning particle size, polydispersity index, refractive index, and viscosity and were chosen for further analysis.

The experiment assessing the solubility of the 5 formulations demonstrated that formula 31, which consists of 14.96% Triacetin, 39.89% Tween 80, 19.94% PEG 400 and 25% water, with oil to surfactant/co-surfactant ratios of (2:8) and a surfactant/co-surfactant ratio of (2:1), exhibited the highest solubility among all five formulas. This formulation yielded a solubility of ATV of 3.95 ± 0.349 ± mg/ml and EZE of 3.04 ± 0.062 mg/ml. The physical properties of formula 31 were found to be as follows: a droplet size of 74.15±1.68 nm, a polydispersity index of 0.337±0.022, a refractive index of 1.3708, and a viscosity of 310 ± 9.58 cP as determined in the study.

The formulas have also been subjected to stability studies both physical and accelerated, except for formula 12 which had the lowest solubility. The microemulsion formulations remained stable throughout the study in terms of droplet size, visual appearance, and assay.

Assay results at time 0 were as follows, for Atorvastatin: ME#22 = 103.5%, ME#26= 99.4%, ME#31= 104.6%, ME#35= 102.5%, whereas, assay of Ezetimibe was: ME#22 = 106.5%, ME#26= 102.5%, ME#31= 104.9%, ME#35= 107.6%.

Dissolution is the rate limiting step of class II drugs, which has low solubility and high permeability, the dissolution rate is directly proportional to the solubility of the drug. Formula 31 has been subjected to in vitro release dissolution profile test, while the other formulas were subjected to dissolution testing. The optimized test conditions for formula 31 were achieved under sink conditions with USP apparatus 2 at a paddle rotation speed of 75 rpm and 900 ml at three different pH (1.2, 4.5 and 6.8). Although the FDA-recommended approach includes SLS and Tween for evaluating dissolution, surfactants were not utilized in our examination as the formulas already contain Tween.

Dissolution results in comparison to Atozet Brand drug, showed that our formula had faster dissolution rates at time points of 5, 10, 15 min, in all tested media of pH=1.2, 4.5 and 6.8.

الملخص

الأتورفاستاتين والإزيتيميب هما عقاران لهما توافر حيوي منخفض عن طريق الفم (14% و35% على التوالي)، وتصفية عالية قبل الوصول الى الدورة الدموية، و/أو استقلاب أولي واسع النطاق، وذلك نظرًا لأن كلا الدواءين يندرجان ضمن تصنيف المستحضرات الصيدلانية الحيوية من النوع الثاني.

من بين علاجات الوقاية الأولية والثانوية من الكولسترول، 3 مثبطات الإنزيم المساعد هيدروكسي -3 ميثيل غلوتاريل(HMG-CoA) A ، أو الستاتينات، تمنع تخليق الكوليسترول في الكبد. بينما يعمل الإيزيتيميب (EZE) كمثبط لامتصاص الكوليسترول.

ومع ذلك، فإن دمج كلا العقارين في دواء واحد يمثل تحديًا كبيرًا نظرًا لاختلاف خصائصهما الفيزيائية والكيميائية..

يشتمل هذا البحث على نظام توصيل الدواء ذاتي الاستحلاب في كبسولة هلامية تحتوي على مادتي الأتورفاستاتين مع الإيزيتيميب ويتمثل النطاق الأساسي لهذه الدراسة في زيادة التوافر البيولوجي للمادتين.

تضمنت هذه الدراسة التطوير الصيدلاني، بالإضافة إلى التوصيف الفيزيائي والكيميائي، وتقييمات الثبات، ودراسات الذوبان في المختبر.

تم تصميم التركيبات المحتوية على ATV/EZE باستخدام 10 مجم من الايزيتيمايب و 40 مجمم من الاتورفاستاتن ,حمض الأوليك أو Triacetin كالطور الزيتي، جنبًا إلى جنب مع المواد الخافضة للتوتر السطحي Tween 80 أو Tween .20 أو Kolliphor® RH40 والمواد الخافضة للتوتر السطحي PEG 400 أو البروبيلين غليكول.

من بين جميع التركيبات التي تم فحصها - باستخدام مخططات الطور الثلاثي الزائف - وجد أن 45 منها لها تكوين أحادي الطور واضح وشفاف. بعد ذلك، نجحت خمس من هذه التركيبات (ME#12، ME#22، ME#26، ME#31، ME#35)في استيفاء المتطلبات الفيزيائية المتعلقة بحجم الجسيمات، ومعامل التشتت المتعدد، ومعامل الانكسار، واللزوجة.

أظهرت تجربة تقييم قابلية ذوبان للتركيبات الخمس المختارة أن التركيبة رقم 31، والتي تتكون من 14.96% Triacetin، 39.89% توين 80، 19.94% PEG 400 و25% ما، مع نسب زيت: مادة خافضة للتوتر السطحي+مادة مساعدة لخفض للتوتر السطحي تبلغ 2:8 بينما النسبة بين المادة خافضة للتوتر السطحي :مادة مساعدة لخفض التوتر السطحي 2:1 ,أعلى قابلية للذوبان بين جميع التركيبات الخمس. أسفرت هذه التركيبة عن قابلية ذوبان ATV تبلغ 3.95 ± 0.349 ± مجم / مل و EZEتبلغ 3.04 ± 0.062 مجم / مل. اما الخصائص

الفيزيائية للصيغة 31 فكانت كما يلي: الحجم 74.15 ± 1.68 نانومتر، ومؤشر تعدد التشتت 0.337 ± 0.022، ومعامل الانكسار 1.3708، ولزوجة 310 ± 9.58 cP.

خضعت التركيبات أيضًا لدراسات الاستقرار الفيزيائية والمتسارعة، باستثناء الصيغة 12 التي كانت ذات قابلية ذوبان أقل. ظلت تركيبات المستحلبات الدقيقة مستقرة طوال فترة الدراسة من حيث الحجم والشكل وكمية المادة الفعالة.

كانت نتائج الفحص في الوقت 0 كما يلي، بالنسبة للأتورفاستاتين: ME#22 = 103.5%، ME#26= 99.4%، ME#31= 104.6%، ME#35= 102.5%، بينما كانت للايزيتيمايب ME#22 = 106.5% ، ME#26= 102.5%، ME#31= 104.9 ME, #35= 107.6%.

الذوبان هو الخطوة التي تحدد معدل الامتصاص في أدوية الفئة الثانية والتي تتميز بقابلية ذوبان منخفضة ونفاذية عالية، يتناسب معدل الذوبان بشكل مباشر مع قابلية ذوبان الدواء. تم عمل الفحص فيما يتعلق بالتركيبة 31 باستخدام جهاز USP 2 بسرعة دوران 75 دورة في الدقيقة و900 مل حجم الميديا في 0.01 مولار من محلول الأسيتات على 3 درجات من الرقم الهيدروجيني (الرقم الهيدروجيني = 1.2 و4.5 و6.8). على الرغم من أن الطريقة الموصى بها من قبل إدارة الغذاء والدواء يتضمن استخدام المواد الخافضة للتوتر السطحي SLS وTween، إلا أنه لم يتم استخدامها في فحصنا لأن التركيبات تحتوي بالفعل على .Tween

أظهرت نتائج الذوبان بالمقارنة مع عقار أتوزت، أن صيغتنا لديها معدلات ذوبان أسرع عند نقاط زمنية تبلغ 5، 10، 15 دقيقة، في كل الاوساط التي تمت دراستها على رقم هيدروجي 1.2 و 4.5 و . 6.8

Chapter 1

Introduction

1. Overview

1.1 Oral route of administration

Oral administration is preferred over other administration routes, but only drugs with sufficient solubility in water and permeability across gastric mucosa can be administered orally [1]. Approximately 9% of new drug entities belong to Class-I (high solubility-high permeability) of the Biopharmaceutics Classification System (BCS), and newest drug molecules have poor solubility in water, resulting in poor oral bioavailability. Formulating poorly water-soluble compounds as oral dosages is the most difficult problem [2].

The poor aqueous solubility of BCS class-II and -IV drugs limit their oral bioavailability because of the limited solubility and slow dissolution rates. Therefore, there is a need to develop novel pharmaceutical formulations to improve the poor solubility of drug molecules to improve their efficacy and increase their commercial viability [3]. However, poor solubility is mainly associated with the chemical structure of the drug molecule because a change in chemical structure often leads to a change in the physical properties of the drug molecule which may lead to improved solubility. Hence, the first step is to modify the chemical structure of the drug molecule to improve its solubility before developing a drug formulation to improve its bioavailability [4], [3].

1.2 Cholesterol

An essential component of human heart health is cholesterol, a waxy substance obtained from animal liver or derived from diet. Cholesterol is used to make hormones, vitamin D, and certain types of bile necessary for digestion. Cholesterol synthesis occurs via both exogenous and endogenous pathways, with the liver being responsible for producing the majority of the body's cholesterol [5].

However, high levels of cholesterol in the blood can lead to cardiovascular disease and other health complications. To manage high cholesterol levels, lifestyle changes such as increased physical activity and a healthy diet that is low in saturated fats are recommended. In cases where lifestyle changes are insufficient, medications such as statins may be prescribed to block the mevalonate pathway of cholesterol synthesis and lower blood cholesterol levels. Moreover, various cholesterol precursor sterols regulate intracellular cholesterol homeostasis, and different cholesterol intermediates and metabolites have diverse roles in the pathophysiology of cholesterol-related disorders, such as familial hypercholesterolemia and atherosclerosis [5].

Cholesterol is categorized as high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Accurately measuring and managing cholesterol levels, particularly LDL-C is crucial, since it increases risk of cardiovascular disease, whereas increased HDL-C is associated with a reduced risk. HDL-C works by removing excess cholesterol from arterial walls and transporting it back to the liver for processing. Therefore, focusing on increasing HDL-C levels while decreasing LDL-C levels should be a key aspect of managing cholesterol and reducing the risk of cardiovascular disease [2], [6].

1.3 Micro-emulsions

A micro-emulsion is a thermodynamically stable, isotropic mixture of oil, water, surfactant and co-surfactant. It is a clear, transparent liquid with droplet sizes typically in the range of 10 to 100 nanometers. The utilization of micro emulsions is feasible for hydrophilic drugs since they can be dissolved in the aqueous phase, also providing an excellent vehicle for delivering lipophilic compounds by dissolving them either in oil or a mixture of oil and surfactant, resulting in enhanced bioavailability and efficacy [7].

Micro-emulsions have a high degree of stability, therefore, micro-emulsions have garnered interest in the pharmaceutical industry as a potential delivery system for drugs targeted at lipid-related disorders such as dyslipidemia [8].

The crucial characteristics of micro emulsions include small droplet size, thermodynamic stability, transparency, ease of preparation, and high drug-loading capacity. In addition, micro emulsions have the potential to improve drug absorption and bioavailability.

Also, microemulsion exhibits transparency due to the droplet size being less than 25% of the wavelength of visible light. These properties make them promising candidates for drug delivery systems [9].

While micro emulsions have many advantages, there are also some disadvantages to consider. One such disadvantage is the potential for toxicity caused by high concentrations of surfactants and co-surfactants, which are required to maintain thermodynamic stability and small droplet size. Another disadvantage is the potential for phase separation, which can occur if the system is exposed to extreme temperatures or pH conditions.

1.3.1 Composition of micro emulsion

1.3.1.1 Oil component

The selection of an appropriate oil component in microemulsions is crucial for achieving optimal physical and chemical properties, drug solubility, and therapeutic efficacy. Moreover, the oil component plays a vital role in determining the safety and biocompatibility of micro emulsions. For instance, certain oils can facilitate drug absorption and enhance therapeutic efficacy by increasing the solubility of lipophilic drugs, while others may result in undesirable side effects or poor drug delivery performance due to their physicochemical properties.

Each type of oil has distinct physicochemical properties, which can impact the droplet size and stability of the micro emulsion as well as affect drug solubility, release rate and bioavailability. For example, long-chain fatty acids can increase the viscosity and droplet size of micro emulsions, while medium chain triglycerides (MCTs) have low viscosity and can improve drug solubility and permeability [10].

1.3.1.2 Aqueous phase

The aqueous phase in micro emulsions generally consists of water and may contain electrolytes, pH modifiers, or other additives to improve the stability and performance of the formulation [10], [11].

1.3.1.3 Surfactants

Surfactants are another crucial component of micro emulsion formulations. They play a significant role in stabilizing the emulsion droplets by reducing interfacial tension and preventing coalescence. Moreover, surfactants can also influence the droplet size and zeta potential of micro emulsions, thereby affecting their stability and drug delivery performance. The choice of surfactant can also impact the biocompatibility and safety of micro emulsions, as some surfactants may cause irritation or toxicity, particularly for topical and transdermal drug delivery applications. Therefore, it is essential to carefully evaluate the biocompatibility and toxicity of surfactants before selecting them for formulations, particularly for pharmaceutical applications [12], [13].

Surfactants are classified as nonionic, anionic, cationic, and zwitterionic surfactants. Nonionic surfactants are widely used in pharmaceutical applications due to their low toxicity and biocompatibility. Anionic and cationic surfactants are less frequently used due to their potential toxicity and limited biocompatibility, while zwitterionic surfactants offers promising approach in improving the stability and biocompatibility of micro emulsions, though their use is less common compared to nonionic surfactants.

1.3.1.4 Hydrophilic-lipophilic balance (HLB)

HLB is a measure of the relative hydrophilic and lipophilic character of a surfactant, which influences its ability to stabilize micro emulsions. Each surfactant possesses a HLB value ranging from 0 to 20. A value of 0 implies oil solubility and low water solubility, while a value of 20 indicates complete water solubility and no oil solubility [14].

Surfactants with a high HLB are more hydrophilic and tend to form stable oil-in-water emulsions (O/W), while those with a low HLB are more lipophilic and better able to stabilize water-in-oil (W/O) emulsions [8],[15], [16].

1.3.1.5 Co-surfactants

Co surfactants are small molecules that work cooperatively with surfactants to improve stability and reduce interfacial tension between the oil and water phases in a microemulsion. Surfactants consisting of only one chain are not enough to effectively decrease the interfacial tension [14]. So, together with surfactants, co-surfactants help to stabilize the micro-emulsion and maintain its thermodynamic stability. Those co-surfactants are typically small alcohols or glycols, Common co-surfactants used include short-chain alcohols such as ethanol and propylene glycol, as well as polyethylene glycol (PEG) and its derivatives. The addition of co-surfactants can also impact the HLB value and droplet size distribution, which should be carefully considered during the formulation process to ensure optimal drug delivery performance [17].

1.3.2 Types of micro-emulsion

There are several types of micro emulsions, including oil-in-water (O/W), water-in-oil (W/O), bi-continuous and multiple emulsions. Oil-in-water (O/W) are the most commonly used type, in which

oil droplets are dispersed within a continuous water phase to improve the solubility, bioavailability and therapeutic efficacy of hydrophobic drugs. Water-in-oil (W/O) are less commonly used and involves the dispersion of water droplets within an oil phase, which can protect the drug from degradation, improve drug stability and reduce toxicity. Bi-continuous and multiple emulsions are more complex types, involving the formation of multiple interpenetrating phases [18].

Their use in drug delivery is still being investigated and optimized, and they have the potential to improve drug targeting, sustained release profiles, and controlled drug release in a range of therapeutic application.

Numerous techniques are available for identifying the two categories of emulsions. The initial approach for testing is known as the dying technique, where a powder-based dye that can dissolve in oil (such as Sudan III) is evenly spread over the emulsion. Subsequently, an examination of the emulsion under a microscope would reveal a red backdrop indicating W/O type, while red distinct dots signify O/W type.

The dilution method is another technique for identifying the type of emulsion. To do this, a sample of the emulsion is mixed with both oil and water.

O/W emulsions disperse quickly in water while W/O emulsions are easily dispersed in oil. Additio nally, electrical conductivity can be measured to distinguish between the two types; O/W has higher conductivity than W/O.

Its's worth noting that during phase inversion, viscosity changes occur suddenly. When adding an aqueous liquid to an existing emulsion, the viscosity of O/W will decrease whereas that of W/O will increase [18].

1.3.3. Preparation of micro emulsion

1.3.3.1 Pseudo-ternary phase diagram

One commonly used method for the formulation of micro emulsions is the use of a pseudo-ternary phase diagram. Pseudo ternary phase diagrams are essential for determining the micro emulsion region and ideal composition for self-micro emulsifying drugs [18].

The technique involves mixing a surfactant, co-surfactant, oil and water in varying proportions to create different formulations with different HLB values and then plotting these formulations on a triangular graph to identify the areas of stable emulsion formation. This method allows for the selection of an optimal formulation with regards to droplet size and stability, as well as providing insight into the relationship between formulation components and micro emulsion properties.

This approach has facilitated the development of micro emulsion formulations with good physical and chemical stability, high drug loading capacity, optimal particle size distribution and improved bioavailability and therapeutic efficacy.

The categorization of a micro emulsion system as either monophasic or biphasic is based on visual examination.

If the mixture appears cloudy and separate into distinct phases, it is classified as biphasic. Conversely, if the solution remains transparent after agitation, then it is deemed to be monophasic. Additionally, it is deemed that the region of the phase diagram encompassed by the sample points denote/s the micro emulsion domains, as illustrated in Figure 1 below [9].

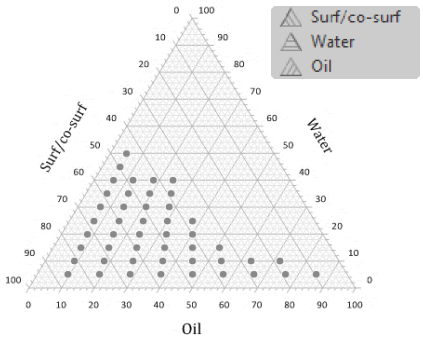


Figure 1: Pseudo ternary phase diagram of microemulsion preparation [9].

1.3.1.1 Phase titration method

Phase titration method is another commonly used technique for the formulation of micro emulsions. Spontaneous emulsification depicted with the help of phase diagrams. By mixing of all components at once and dilution of an oil-surfactant mixture with water to make w/o micro emulsion or dilution of a water surfactant mixture with oil to make o / w micro emulsion**.** The stability of the system can be determined by observing changes in droplet size and turbidity, as well as by monitoring for phase separation. This method allows for the identification of the optimal ratio of components needed to form a stable micro emulsion system [14]. Figure 2 below explains the method in short steps.

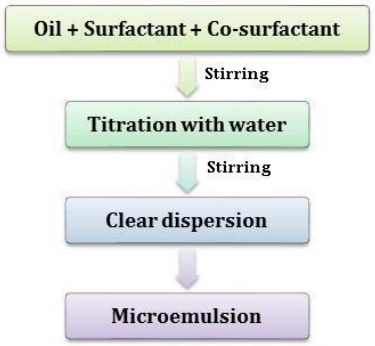


Figure 2: Step by step of phase titration method [13].

1.3.3.3 Phase inversion method

Another widely used method for creating micro emulsions is the phase inversion method. This method involves the formation of a single-phase, water-in-oil (W/O) or oil-in-water (O/W) emulsion, followed by the inversion of phase to form a stable micro emulsion. This can be achieved by changing the ratio of the surfactant to oil or water and adjusting the pH of the system [14]. The phase inversion method can also be used in combination with a titration procedure to optimize the composition of the formulation and achieve the desired stability and properties of the final emulsion product. To create a pseudo ternary phase diagram using the phase inversion method, different ratios of oil, water and surfactant (and co-surfactant) are mixed and stirred until a homogeneous mixture is achieved. This mixture is then titrated with one of the components, typically water or oil, to induce phase separation and form a micro emulsion. This process is repeated for each composition, and the resulting data points are plotted to determine the boundaries of the micro emulsion region in the phase diagram. The phase inversion method is particularly useful for producing micro emulsions with a narrow droplet size distribution, high loading capacity of oil-soluble drugs, and excellent stability. As can be seen in Figure 3 below explaining the process of inversion from W/O emulsion to O/W emulsion.

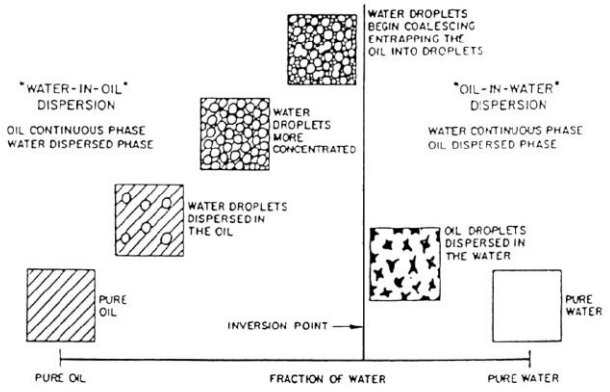


Figure 3: Phase inversion Process Form W/O emulsion to O/W emulsion [19].

1.3.4 Factors affecting microemulsion

There are several factors that can influence the formation of micro emulsions. These factors include the choice and ratio of oil, water, surfactant and co-surfactant, as well as the temperature, mixing speed, and type of mixing method used. The choice and ratio of oil, water, surfactant and co-surfactant have a significant role in determining the size distribution and stability of micro emulsions. In addition, the pH and ionic strength of the system can also affect the properties of the emulsion. Furthermore, the choice of mixing method and mixing speed can influence droplet size distribution, as well as physical and chemical stability. Therefore, the optimization of these factors is crucial in creating stable and high-quality micro emulsions. Some of the methods for optimizing these factors include using statistical experimental design and response surface methodology to determine the optimal conditions that result in stable Micro-emulsions , as well as using high-pressure homogenization, microfluidics, and ultrasound methods to improve the emulsion properties [12], [13].

1.3.4.1 Temperature

Temperature is one of the factors that can affect the formation and properties of microemulsion. Various studies have shown that temperature can affect droplet size distribution, interfacial tension, and viscosity of the system during emulsion formation. Furthermore, at higher temperatures, droplet size tends to decrease due to lower viscosity and increased Brownian motion. On the other hand, at very high temperatures, coalescence and Ostwald ripening may occur which can lead to droplet growth and aggregation [12], [13], [20].

1.3.4.2 Packing ratio

The packing ratio, which refers to the ratio of oil volume to surfactant (and co-surfactant) volume

in the system, is a critical parameter that can significantly affect the formation and stability of Micro-emulsions. Packing ratio values that are too low may cause instability and separation of the emulsion, while values that are too high can lead to difficulty in droplet formation and larger droplet sizes [10], [12], [13], [20].

1.3.4.3 Nature of surfactant and co-surfactant

The nature of surfactant and co-surfactant used can have a significant impact on the formation and stability of microemulsion. The size of the lipophilic tail group and hydrophilic head group in surfactants play a crucial role in determining their effectiveness for specific formulations. These measurements determine how much oil can expand the tail area and how water can swell the head group, which is important when estimating a surfactant's HLB value for a particular system. Hence, these properties are significant considerations while developing methods to utilize dispersed lipid formulations that do not require potentially toxic surfactants [11].

1.3.4.4 Chain length

It has been found that shorter chain length surfactants tend to form smaller droplets and more stable micro emulsions as compared to longer chain length surfactants.

This is because shorter chain length surfactants can more easily form a dense interfacial film, which helps to stabilize the emulsion. On the other hand, longer chain length surfactants tend to form more viscous interfacial layers, which can lead to coalescence and instability of the emulsion.

Regarding the effect of co-surfactants on chain length, the inclination towards (w/o) can be enhanced by co-surfactants that possess longer chains, whereas those containing shorter chains have a tendency to boost hydrophilicity and support (o/w) [14].

1.3.4.5 Property of oil phase

The nature of the oil used, including its hydrophobicity and chain length of fatty acid residues, can strongly influence droplet size and stability of the resulting micro emulsion. Additionally, the presence of impurities and antioxidants in the oil can also impact overall emulsion properties.

Moreover, the oil can also affect the biocompatibility and toxicity of micro emulsions. For example, soybean oil and medium chain triglycerides (M.C.T) oil are commonly used due to their biocompatibility and low toxicity, while some oils such as castor oil are known to exhibit toxic effects and are therefore not recommended for use in biomedical applications. Furthermore, the properties of the oil phase can also affect drug-loading capacity and release kinetics of micro emulsions.

1.3.4.6 Water content

An increase in water content can lead to a decrease in droplet size, as well as an increase in zeta potential and stability. However, adding too much water can lead to phase separation and instability of the micro emulsion.

1.3.4.7 pH

The pH of the micro-emulsion system can influence droplet size and stability. A change in pH can affect the surface charge and hydrophobicity of the surfactant, which can impact its interfacial activity and ultimately the stability of the micro-emulsion. It is essential to carefully control the pH of the micro-emulsion system during its preparation and storage, as even minor pH changes can affect drug encapsulation, stability and bioavailability. Furthermore, the pH of the micro-emulsion can also have a significant impact on drug release kinetics.

1.3.4.8 Salinity

Salinity impacts the stability of micro-emulsions. In some cases, high levels of salt can destabilize the micro-emulsion system and cause droplet aggregation or coalescence.

This is due to the ability of salt ions to compete with surfactants for adsorption at the oil-water interface, reducing its stabilizing effect. However, in other cases, salinity can enhance the stability of micro-emulsions by increasing the surfactant's interfacial activity and reducing the thickness of the electrical double layer.

1.3.5 Characterization of microemulsion

The characterization of micro-emulsions is crucial for understanding their physical and chemical properties, including droplet size distribution, zeta potential, viscosity, and transparency. Droplet size distribution can be measured using techniques like dynamic light scattering, while zeta potential can be determined by a particle analyzer. Viscosity can typically be measured using a viscometer or rheometer. Transparency, which is an indicator of clarity and uniformity of the micro-emulsion, can be visually assessed. In addition to these basic physical and chemical characteristics, other properties such as drug loading capacity, drug release kinetics, and toxicity should also be evaluated [9], [10], [21].

1.3.5.1 Droplet size distribution

Droplet size distribution of microemulsion can be measured using techniques like dynamic light scattering, which involves the analysis of scattered light to determine particle size and distribution.

Works by analyzing the intensity fluctuations of scattered laser light caused by Brownian motion of particles. This technique can measure the size of particles ranging from a few nanometers to several microns, making it ideal for determining droplet sizes in micro-emulsions. Moreover, dynamic light scattering provides information on the polydispersity index, which is a measure of the

width of size distribution [22], [23].

In addition to dynamic light scattering, there are other scattering techniques that can be used to determine droplet size in microemulsions, such as static light scattering and electrophoretic light scattering. Static light scattering is another technique that measures the scattered light from particles, but it does not require time-dependent measurements. Instead, it relies on the angle dependence of scattered light intensity to determine particle size [22]–[26].

1.3.5.2 Zeta potential

Zeta potential is an important parameter that can affect the stability and behavior of micro-emulsions. By measuring the zeta potential of microemulsions using electrophoretic light scattering, it is possible to gain insight into their stability and behavior in different conditions.

The method involves applying an electrical field to the sample and measuring the movement of charged particles in response to the electric current. The zeta potential can be calculated from the electrophoretic mobility of the particles using the Smoluchowski equation, which relates particle charge to the speed of particle movement. In addition to electrophoretic light scattering, there are other techniques that can be used for zeta potential measurements, such as laser Doppler velocimetry and phase analysis light scattering, but electrophoretic light scattering is particularly suitable for micro-emulsions due to its ability to measure the zeta potential of small particles and low sample volumes [8], [10][27].

1.3.5.3 Measuring viscosity of micro emulsion

Viscosity measurements of micro emulsions can be conducted using various techniques, including rotational viscometer and capillary viscometer. Rotational viscometer involves measuring the torque required to rotate a spindle at a certain speed in the sample, which is related to the viscosity of the sample. Capillary viscometer involves measuring the time required for a known volume of liquid to flow through

a capillary under controlled conditions, which is also related to the viscosity of the sample. Both techniques have advantages and disadvantages, but rotational viscometer is more commonly used for micro emulsion viscosity measurements due to its wider range of shear rates and its ability to measure viscosity over a broader temperature range. Additionally, rheological measurements can also provide valuable information about the behavior of Micro-emulsions under different conditions and can be used to characterize their flow properties and stability [8], [10].

1.3.5.4 Transparency

The transparency of micro emulsions can be measured using spectrophotometry, which involves shining a beam of light through the sample and measuring the amount of light that is transmitted through it. The transparency of micro emulsions is influenced by their composition, size and shape of the droplets, and refractive index mismatch between the different phases present in the micro emulsion. Transparency can also be affected by changes in temperature and pH, as well as the presence of impurities or contaminants [8], [12].

1.3.5.5 Electron microscopy

Electron microscopy is a powerful imaging technique that allows for visualization of the microstructure and morphology of micro emulsions.

This technique can provide information about the size, shape, and distribution of droplets in the micro emulsion, as well as any changes that may occur due to external factors such as temperature, pH, and shear stress. In addition to providing qualitative information about the micro emulsion structure, electron microscopy can also be used for quantitative measurements of droplet size and distribution, which can provide valuable insights into the stability and performance of the microemulsion. Another advantage of

electron microscopy is its ability to capture images at high magnification and resolution, which can reveal details that are not visible using other techniques [10].

Electron microscopy can be used to differentiate micro emulsions and macro emulsions. Microemulsions have droplet sizes ranging from 10 to 100 nm and are thermodynamically stable, while macro emulsions have much larger droplet sizes, typically ranging from 100 nm to several microns, and are kinetically stable. Microemulsions are optically transparent, whereas macro emulsions can be opaque due to the scattering of light by larger droplets. Microemulsions also have a lower interfacial tension, higher solubilization capacity and better drug release properties compared to macro emulsions [18].

Furthermore, microemulsions can penetrate biological barriers more easily due to their small droplet size, making them desirable for transdermal and oral drug delivery applications.

1.3.5.6 Stability studies of micro emulsions

Accelerated stability studies for micro emulsions involve subjecting the samples to stress conditions such as high temperature, freeze-thaw cycles, and agitation [2].

Freeze-thaw cycle testing involves subjecting the microemulsion to multiple cycles of freezing and thawing in order to evaluate its physical stability [28][29].

Performing freeze thaw cycle testing involves freezing the microemulsion at a temperature below its freezing point, followed by thawing at room temperature or higher temperatures. The number of cycles and the duration of each cycle can be adjusted based on the specific application and storage conditions to which the microemulsion will be exposed [10].

1.3.5.7 Centrifugation stress testing

This involves subjecting the microemulsion to centrifugal force to assess the droplet size distribution, sedimentation, and creaming behavior of Micro-emulsions [30], [31].

1.3.5.8 Long term stability studies for microemulsion

Long-term stability studies involve subjecting the microemulsion to various storage conditions, such as different temperatures and humidity levels, for an extended period of time by storing the formulas under ambient conditions and testing after 1, 3, and 6 months [8], [10].

1.3.5.8.1 Thermal stability

These methods allow for the evaluation of changes in microemulsion structure and composition under different temperature conditions, providing critical information necessary for optimizing storage and application conditions of micro emulsions.

In order to assess the thermal stability of micro emulsions, differential scanning calorimetry and thermogravimetric analysis are commonly used techniques. Differential scanning calorimetry involves heating the microemulsion sample at a controlled rate while measuring its heat flow. The resulting data can be used to determine the material's thermal properties, such as its melting and crystallization temperatures [32], [33]. Thermogravimetric analysis involves subjecting the microemulsion to a controlled temperature ramp while measuring its weight loss [34], [35]. Both techniques allow for the evaluation of thermal stability by detecting any changes in microemulsion structure and composition as a function of temperature [8], [10].

1.3.5.9 Determination of pH of the microemulsion

The pH of a microemulsion can also play an important role in its effectiveness and application. pH determination can be performed using a pH meter or indicator strips, and the optimal pH range for Micro-emulsions may vary depending on their specific application [11].

1.3.5 Differences between a microemulsion and others

1.3.6.1 Microemulsion vs. Macroemulsion

A key distinction between macro emulsion and microemulsion lies in their particle sizes. While the drop size of macroemulsion typically falls within the range of 0.5 μm to 500 μm, due to the force of gravity, these droplets have a tendency to settle [36]. Additionally, macro emulsions are classified as thermodynamically unstable systems based on their positive interfacial free-energy levels [37],[38],[39].

Macroemulsion storage can lead to a variety of phenomena such as phase inversion, flocculation, phase separation, coalescence and creaming. On the other hand, microemulsions are optically clear (transparent), have much smaller particles (in the range of 100nm to 600nm) due to their thermodynamically stable colloidal dispersion structure formed by two immiscible liquids coexisting in one phase with surfactant and co-surfactant molecules that balance hydrophilic-lipophilic properties [40].

Microemulsion is characterized by its extremely low interfacial tension (almost 0), high interfacial area and absence of interfacial free energy [8], [41], [42].

1.3.6.2 Micro-emulsions are not Nano emulsions

In terms of composition, Microemulsion and Nanoemulsion differ in their method of formation. Microemulsions are emulsified at the nanoscale through self-assembly mechanisms while intense mechanical shear is necessary to form Nano scale emulsion or simply “Nanoemulsion” [43].

Microemulsion can be produced by employing high concentration of surfactant approximately 40% while gently agitating. Self-assembly at the Nano-scale is achieved by utilizing a significantly high quantity of surfactants. Bowcott Schulman's research has demonstrated that self-emulsification is achievable under the condition of zero interfacial tension between oil and water [44].

The interfacial tension is expressed as follows:

$$\gamma_i = \gamma_{ow} - \pi \quad \text{……………………………………………………………. (1)}$$

γ_{ow} = The interfacial tension without the presence of surfactant.

π = The spreading pressure of surfactants at the interface.

The quantity of added surfactant is directly proportional to π value. As a result, interfacial tension can become negative when the π exceeds γ_{ow}. Microemulsions exhibit a high level of stability due to their negative free energy, which is caused by the presence of a negative interfacial tension. When the interfacial tension is positive, coarse emulsions are more likely to form when $\pi < \gamma_{ow}$. These types of emulsions have droplets that tend to coalesce together over time.

In order to form Nanoemulsions, high levels of mechanical shear are required to break down large droplets into much smaller ones. This process demands an intense level of shearing force that can effectively overcome the significant interfacial tension between the phases involved. Nanoemulsions differ from Microemulsions in that they are not thermodynamically stable systems due to the high interfacial tension between their oil and water phases [45].

1.3.5.3 Micelles

Upon surpassing a specific concentration level, surfactant molecules congregate to create aggregates known as micelles. The point in which the minimum required surfactant concentration is attained for constructing these micelles structures is called the critical micelle concentration. The distribution of surfactant molecules is thermodynamically advantageous. Within an aqueous environment, the hydrophilic portions of surfactants are enclosed by water molecules while the hydrophobic tails congregate in the micelle's core. The micelles in oil consist of surfactant molecules with their hydrophilic heads located inside them, while the hydrophobic tails extend from the core towards the oil phase. This is known as reverse micelles [41].

Micelles and emulsions differ mainly in terms of their liquid phase. Micelle formation relies on the addition of surfactants to a single liquid component, which can either be oil (reversed micelles) or water. Emulsions, however, are prepared using a dual-liquid system by adding surfactants to two immiscible liquids such as soybean oil and water [46].

Micelles display an exceptional inner configuration wherein the nonpolar tails of surfactant molecules amass exclusively at the core center. As soon as the surfactant concentration surpasses its critical micelle concentration, a fraction of oil droplets is enabled to permeate through the hydrophilic "shield" encircling micelles, thereby getting stabilized in their central region.

As a consequence of the penetration process, there is an expansion in the interfacial area which subsequently leads to higher surfactant spreading pressure at the interface [47].

According to equation 1, microemulsions can be thermodynamically stable when the spreading pressure of surfactant at the interface π is higher than without its presence, resulting in a small oil-water interfacial tension, $\gamma_{i,}$ close to zero.

Schematic representation representing the difference of the dispersed phase structure of micelles, reverse micelles, o/w microemulsion and w/o microemulsion is shown in Figure 4 below.

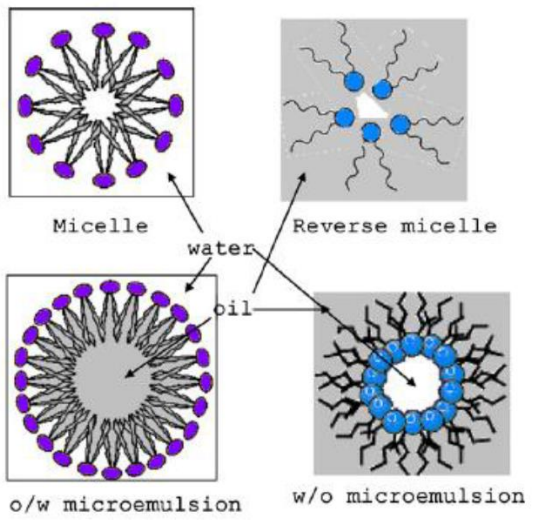


Figure 4: Schematic representation of the dispersed phase structure of micelles, reverse micelles, o/w microemulsion and w/o microemulsion [18].

1.4 Atorvastatin:

1.4.1 Description

Atorvastatin (ATV), defined chemically as ([R-(R*, R*)]-2- (4-uorophenyl)-dihydroxy-5-(1-methylethyl)-3-phenyl-4 [(phenylamino)carbonyl]-1H-pyrrole heptanoic acid, hemi-calcium salt).[2], [6], [10]. The chemical structure of the Atorvastatin is shown in Figure 5 below.

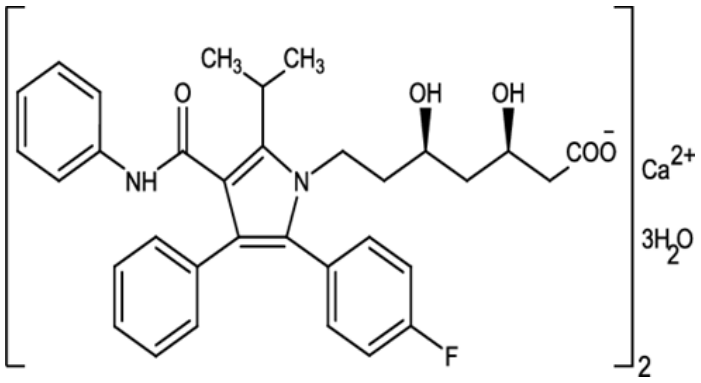


Figure 5: Chemical structure of Atorvastatin [48]

1.4.2 Biopharmaceutical Classification System (BCS)

Atorvastatin is a class–II compound of BCS, with poor solubility in water and high presystemic clearance.

1.4.3 Mode of action

Because Atorvastatin inhibits hydroxymethyl glutaryl-coenzyme A (HMGCoA) reductase selectively and competitively, it is commonly prescribed for treating high blood cholesterol. During sterol biosynthesis, mevalonate is converted from HMG-CoA, which is the rate-limiting step. By inhibiting ATR, mevalonate levels decrease, and hepatic cholesterol levels decrease and LDL uptake increases [2], [6].

1.4.4 Solubility

Atorvastatin is freely soluble in methanol [49], slightly soluble in ethanol (96%), very slightly soluble in water, practically insoluble in methylene chloride [2], [6], slightly soluble in distilled water, pH 7.4 phosphate buffer, acetonitrile, insoluble in aqueous solutions of pH 4 and below, which are the conditions typically present in the stomach [49].

1.4.6 Techniques of formulating Atorvastatin

To enhance its solubility and to increase its bioavailability, Atorvastatin was formulated into a sustained-release capsule using conventional micronized and wet granulation techniques. However, these conventional formulation methods yield highly crystalline particles with narrow size distributions that are difficult to process and maintain stability. To overcome these limitations, several novel techniques have been investigated. such as the nanoprecipitation method to precipitate fine nanoparticles from an aqueous solution of Atorvastatin using a nonionic surfactant (Tween 80) [50]. The method was simple, fast, inexpensive, reproducible, and controlled and thus is suitable for large-scale production. This method also reduced drug crystallinity and improved the physicochemical properties of the drug particle including increased particle size, decreased polydispersity index, and improved solubility in simulated gastric fluid and intestinal fluid. In another study, a spray-drying technique was used to form micro sized capsules containing colloidal dispersions of Atorvastatin with a size range of 100-150 nm [51].This technique yielded a bimodal distribution of drug particles and had high encapsulation efficiency, low dosage variability, and good physical stability [51]. Although these techniques have improved the physicochemical properties of Atorvastatin and enhanced its solubility and bioavailability, there are still further improvements that can be made to enhance these properties even further [2], [6].

1.5 Ezetimibe

1.5.1 Description

Ezetimibe (EZE), defined chemically as [1-(4-fluorophenyl)- 3(R)- [3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4 hydroxyphenyl)- 2-azetidinone] [2], [6]. Chemical structure of Ezetimibe is represented in Figure 6 below.

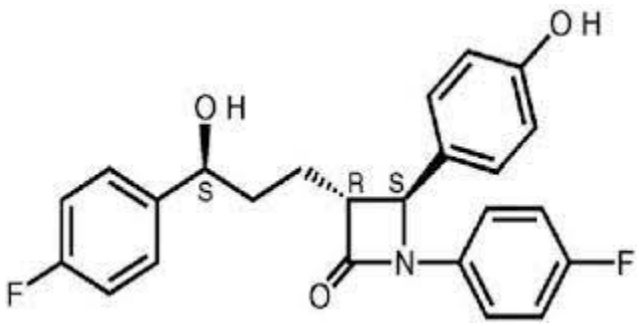


Figure 6: Chemical structure of Ezetimibe [48].

1.5.2 Biopharmaceutical Classification System (BCS)

Ezetimibe is classified as a member of (BCS class-II).

1.5.3 Mode of action

Functions as bile acid sequestrant and works as lipid-lowering compound, blocks cholesterol absorption into the intestines without affecting triglycerides, vitamins, or bile acids.

1.5.4 Solubility

Ezetimibe is practically insoluble in water, soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF)s, freely soluble in acetone. The solubility of Ezetimibe is approximately 15 mg/ml in DMSO and approximately 20 mg/ml in ethanol and DMF [2], [6], [52]. EZE is practically insoluble in aqueous media and the solubility of anhydrous and hydrated forms of the substance is approximately 12 µg/mL and 8 µg/mL, respectively [53].

1.5.6 Techniques of formulating Ezetimibe

This lipid-lowering drug is a weak base that can be formulated as a suspension or a powder for oral administration. Unlike water-soluble drugs such as aspirin or caffeine, the oral suspension of Ezetimibe has a low viscosity. For this reason, it is difficult to prepare the drug as a suspension. Moreover, it suffers from poor stability and rapid sedimentation of the crystals formed when the suspension is exposed to light. In order to overcome these challenges, researchers have developed a new method for formulating Ezetimibe using micro-encapsulation technology [2], [6].

1.6 Self-emulsifying drug delivery systems

Self-emulsifying drug delivery systems represent an isotropic mixture of oils (natural or synthetic), surfactants (solid or liquid), hydrophilic solvents and co-solvents/surfactants, that functions as a delivery system for drugs with an oil base, and upon addition to water or biological fluids spontaneously forms a micro emulsion with droplet sizes ranging from 20-200 nm [18], [54], [55].

These systems create fine o/w micro- and Nano-emulsions spontaneously when diluted with GI fluids and emulsify upon mild agitation. So, this is known as in situ or self-emulsification.

Due to its ability to self-emulsify into microemulsion easily and consistently under mild stomach conditions, this drug delivery system is particularly well-suited for hydrophobic drugs. Additionally, the pre-mixture can be stored in capsules for an extended period of time thanks to its high thermodynamic stability.

In comparison to other systems, these systems seize abundant features including but not limited to: small droplet size, unique physicochemical properties, increased stability, enhanced permeability and absorption of the drug, reduced side effects, and improved patient compliance. In addition, the self-emulsifying micro

emulsion drug delivery system can be applied to both hydrophobic and hydrophilic drugs while maintaining their therapeutic efficacy [2], [6].

On the other hand, one significant drawback of SMEDDS is the substantial quantity of surfactant necessary for their formation. Typically, four to five times surfactant to oil ratio is needed to create a microemulsion. Additionally, excessive use of surfactants can have toxic consequences. Consequently, there is an urgent need to decrease reliance on these agents while still achieving microem ulsion -level droplet size.

1.7 Soft gel capsules

Soft gel capsules are a popular delivery system for drugs and cosmetic products because they offer several advantages over conventional tablets, such as ease of use, better bioavailability, and improved safety. Soft gels are similar to regular capsules but are made of a soft material such as gelatin instead of a hard plastic. This allows the soft gel to conform to the anatomical contours of the oral cavity, thus facilitating more efficient drug absorption in the stomach [36], [37]. Due to the softness of the material, the soft gel capsule easily dissolves within a few minutes after being swallowed, which results in faster and more complete absorption of the drug it contains. One major advantage of soft gel capsules over tablets and caplets is that they provide controlled release of drugs, which make them particularly useful in special cases such as chronic conditions which necessitate long period of time drug delivery [36].

Soft gel capsules also exhibit a higher bioavailability than tablets and caplets because of their smaller particle size and uniform distribution of drug molecules throughout the polymeric matrix [36], [37].

The inherent lipophilicity of poorly water-soluble drugs leads to poor dissolution in the gastrointestinal tract and in many cases leads to inconsistent absorption and limited clinical effectiveness [56]. The rate

of absorption can be increased by physically protecting them from gastric acidity and other digestive enzymes. The incorporation of these drugs into lipid carriers is the most commonly used method for increasing the rate of absorption and decreasing their adverse effects on the liver [56], [57].

Recently, there is a cumulating interest in using soft gels as drug carriers due to their easy preparation and cost-effective manufacturing process. These small gelatin capsules are filled with non-aqueous liquids containing the active agent dispersed within this carrier that gradually leaks out into the digestive tract[57], releasing the drug over time. In addition to protecting the drug from the acidic environment of the stomach, the soft gel coating allows for prolonged retention in the stomach and delays its passage into the small intestine. This prolongs contact with the intestinal enzymes, thereby improving the rate and extent of absorption and reducing side effects such as gut toxicity [36].

1.7.1 Empo Caps LP+

The Empo Caps LP+ are 100% pharmaceutical grade bovine gelatin capsules, known for their high stability and secure locking mechanism. They are being designed for the encapsulation of fine powders and liquid fills, providing versatility for a critical type of pharmaceutical formulations [58].

These capsules are designed with a pre-lock size and configuration that prevents premature separation during shipping and storage, as can be seen in Figure 7 below, ensuring that they remain intact until filled. They also have a computer-engineered dome radius that reduces denting or dimpling during filling, and the amount of air allowed to escape is optimized for a uniform closed capsule length. Furthermore, these capsules have a double-lock and zone of constant diameter to ensure proper engagement of the cap [58]. Configuration of the caps is represented in Figure 7.

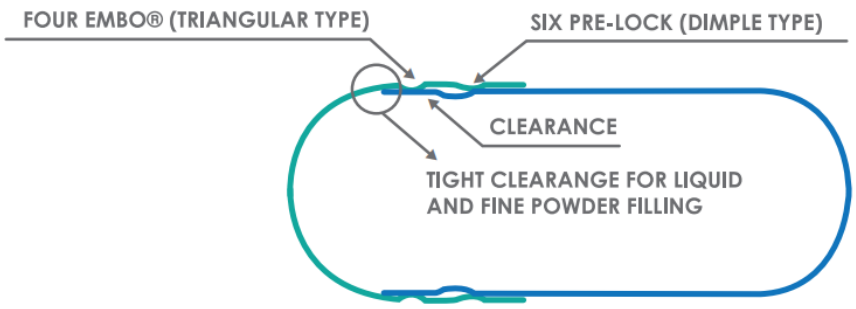


Figure 7: Configuration of EMBO Caps. [59]

1.8 Combining soft gel capsules with SMEDDS

The accustomed method of delivering SEDDS is filling capsules with liquid or dispensing them as oral solutions.

As a result, many drawbacks have been detected including: compromised stability, precipitation of excipients/drugs, as well as volatile ingredients leakage. The use of soft gel capsules to be filled within liquid SMEDDs (L-SMEDDs) is an evolution, transcending previously mentioned drawbacks of SMEDDs. In addition to merging the benefits of the two systems of SMEDDs and soft gels (improved bioavailability solubility, portability, ease of handling, stability and reproducibility) [2], [6].

1.9. Bioavailability and pharmacokinetics

Pharmacokinetics is the study of how drugs are transported throughout the body, which is the actual movement of drugs [60].

Bioavailability is the fraction of a drug that reaches the blood system before it is metabolized and have a positive pharmacological effect on the body [61].

Studies are conducted side by side with metabolism studies to specify half-life elimination, volume of distribution and of course the bioavailability. As mentioned previously, it is an expression that describes the proportion and speed of the active material of a drug which gets absorbed and gets to the blood stream when inserted into the body.

Studies estimates the proportion of the orally administered dosage that gets absorbed into the circulation when compared to the other types of dosage forms like suspensions, solutions or intravenous and provide pharmacokinetic data that is related to the distribution, elimination, proportionality, nutrients effect, and linearity in pharmacokinetics of both the active and inactive moieties.

In addition to all previously mentioned, there are other elements that plays a minor part in the bioavailability, like, metabolism, pH of the stomach and gastric emptying time [61].

Other terms should be taken under consideration, like pharmacodynamics. pharmacodynamics in its simplest definition is how the drug affects the body, while pharmacokinetics is the quiet opposite, its defined as what the body does to the drug.

1.9.2 Pharmacokinetic-dynamic model

Pharmacokinetics uses mathematical equations and models in order to inspect and describe the time course of the drug concentration in the body fluids. Whereas, pharmacodynamics is the part that determines the time course and intensity of the effect of the drugs on the body.

The model usually involves determining the concentration as well as the effect of the drug at multiple time stages after dose administration. The relationship between Pharmacokinetic and pharmacodynamics is determined separately and after that its evaluation is registered both mathematically as well as graphically. Parameters of pharmacokinetics and pharmacodynamics are represented in Figure 8 below.

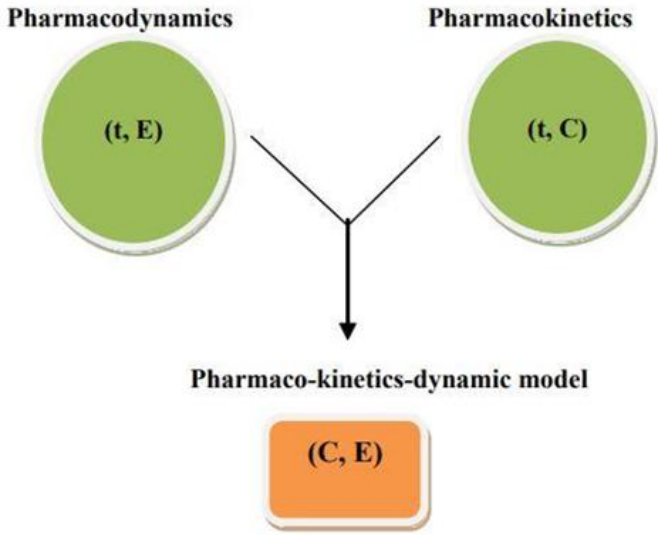


Figure 8: Pharmacokinetics and pharmacodynamics [62]

Bioavailability is assessed using pharmacokinetic parameters or plasma concentrations as well as pharmacodynamics parameters or pharmacological.

However, there is a nonlinear relationship combining the drug's concentration in the blood, pharmacological effects (E), and the active site, this relationship is expressed by Hill's equation. Represented in equation 2. As a result, to this nonlinear relation, the Bioavailability value may differ.

$$E = \frac{Emax * C^n}{EC_{50}^n + C^n} \quad \text{..... (2)}$$

Chapter 2

Problem, Objectives and work plan

2.1 Problem

The low oral bio-availabilities of Atorvastatin and Ezetimibe (14% and 35% respectively) are due to that both drugs are classified as class II of (BCS) and their Presystemic clearance is extremely high, and/or extensive first-pass metabolism.

Despite that the pharmacokinetic profiles of the two drugs are suitable to create a combination. When attempting to incorporate both drugs into a single drug delivery system, some challenges are introduced, such as:

1. Different physicochemical properties. ATR is a weak acid with a solubility of 0.8 mg/mL and pKa of 4.46.6. On the contrary, EZE is practically insoluble and weakly basic compound with solubility of 0.012 mg/mL, and pKa of 9.75.7. Therefore, it may be difficult for both drugs to be solubilized using a single solubilizing agent or strategy (e.g., pH-modifying agent) and successfully developing a formulation will involve the incorporation of more elaborate strategies.
2. Atorvastatin calcium is stable at a basic milieu which is because the basic media inhibits the formation of the lactone impurity. Which is stimulated since the active is prone to oxidation and Moisture-induced degradation.
3. Cellulose is a frequently used excipient with Atorvastatin. Meanwhile, microcrystalline cellulose MCC binds with Ezetimibe resulting in retarded release.
4. Atorvastatin is stable at neutral/alkaline pH, while basic media is detrimental to Ezetimibe.
5. Solubility issues of Ezetimibe
6. Atorvastatin is sensitive to various environmental factors such as heat, moisture, acidic conditions, and light. Under acidic conditions, the hydroxy acid component of Atorvastatin transforms into lactone. Moreover, during the formulation process, Atorvastatin may face additional instability when

it comes into contact with other excipients' molecular components. Given that commonly employed excipients like binders, diluents, anti-adherents, and surfactants can potentially interact unfavorably with Atorvastatin; therefore, including a stabilizer in the composition becomes essential. The stabilizing agent utilized to preserve the Atorvastatin formulation might cause deterioration of Ezetimibe if they directly interact;

After considering the above factors of Ezetimibe and Atorvastatin, attempts were made to formulate a combination in different dosage forms, such as a bilayer tablet comprising ATV as an immediate release layer, and EZE as a sustained release layer, which requires excessive time and effort in formulating and processing [63]. All the methods applied required separating the two actives so that they don't come in contact with each other.

Application of SMEDDS technology to ATR and EZE is a promising strategy to improve their solubilities and bio-availabilities. It has been reported that surfactants commonly used in SMEDDS can inhibit P-gp efflux of various drugs, including EZE. They can also inhibit the activity of numerous CYPs such as CYP3A4, the major metabolizer of ATR. Furthermore, oils used in SMEDDS can enhance lymphatic transport of drugs, bypassing hepatic first-pass metabolism. These properties may be optimal for solubilizing EZE and ATR, ultimately increasing bioavailability of both drugs.

In this research, the effect of encapsulating a SEMDDS in a soft gel capsule on enhancing the bioavailability of the ATR/EZE combination was investigated.

2.2 Objectives

This research comprises a self-microemulsifying drug delivery system encapsulated in a soft gel capsule of Atorvastatin with Ezetimibe. The study's main scope was to increase bioavailability using a soft gel self-micro emulsifying drug delivery system.

In addition, solubility enhancement since it frustrates the rate-limiting step in the case of BCS class II drugs (low solubility and high permeability), drug absorption having more consistent temporal profiles, and drug targeting are eclectic to distinct windows in the GIT.

The following steps were followed in completing the project:

1. An analysis method was developed and validated.
2. Pseudo ternary phase diagrams were prepared and used in the screening process of surfactants, co-surfactants and oils used in the preparation of the SMEDDS
3. 45 formulas had monophasic clear dispersion and were used in further analysis.
4. 5 formulas were subjected to physical testing and stability testing.
5. Dissolution profiles of the Brand drug Atozet with our formulas were constructed and compared.

2.3 Work plan

The diagram in Figure 9 describes each step in the work plan of this thesis.

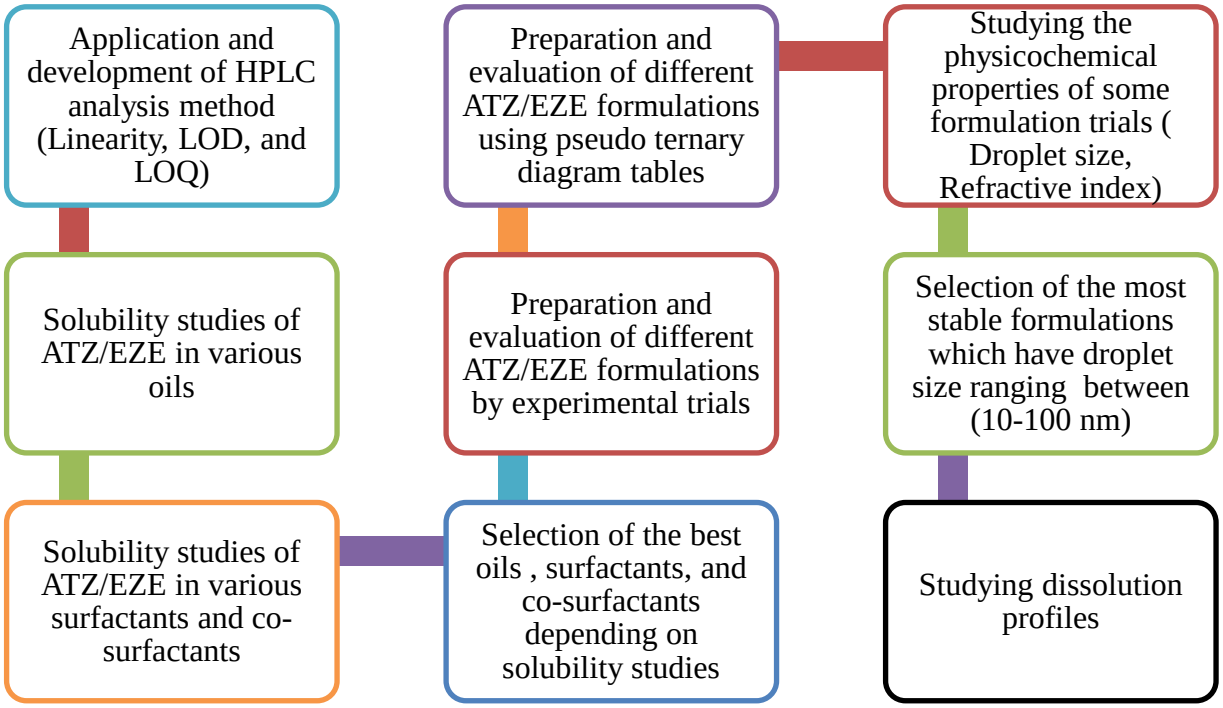


Figure 9: Work plan of the project

Chapter 3

Methodology

3.0 Methodology

3.1 Materials

The reagents and materials used for this study were:

3.1.1 Materials for formulation

Materials used in the study for formulation purposes are depicted in Table #1.

Table 1: Materials for formulation and their function.

Material	Function	Source	Grade
Atorvastatin calcium	API	Cadila pharma	USP
Ezetimibe	API	Teva	USP
Distilled water	Aqueous phase	-	USP
Kolliphor® RH 40	Surfactant	BASF Pharma	USP
Tween 80	Surfactant	Croda	USP
Tween 20	Surfactant	Merck	USP
Propylene glycol®	Co-surfactant	DOW Europe	USP
PEG 400	Co-surfactant	Dow chemic	USP
Ethyl oleate	Oil phase	Croda	USP
Sesame oil	Oil phase	HNRMT	USP
Castor oil	Oil phase	Gustavees	USP
Olive oil	Oil phase	Gustavees	USP
Soybean oil	Oil phase	Gustavees	USP
Oleic acid	Oil phase	Fisher chemical	USP
Triacetin, 99%	Oil phase	Thermos scientific	USP

3.1.2 Materials for analysis

The following materials were used for analysis in this study

Table 2: Reagents used for analysis.

Material	Source	Grade
Methanol	Sigma-Aldrich	Analytical grade
Acetonitrile	Sigma-Aldrich	Analytical grade
Ammonium Acetate buffer	Sigma-Aldrich	Analytical grade

3.2 Equipment and tools

The equipment and tools used in the study are illustrated in Table 3.

Table 3: Equipment and tools

Equipment	Type
HPLC/UV detector	Dionex HPLC (12000 series)
Centrifuge with (BRK5424) Rotor	Lab Tron, Model: LLS-A12
Analytical balance	OHAUS, PIONEER no. ANB002)
Zetasizer DLS	Brookhaven Instrument
PH meter	Mettler Toledo seven multi
Multi magnetic stirrer	VELP Scientific a no. MST019
Hot plate with magnetic stirrer	Thermo scientific
Micropipette	Multi-Volume Single Channel Micropipette
Refrigerator	Beko (BER036)
Bath Sonicator	Elma, S 300H, Elmasonic
Refractometer	KRUSS Optronic GmbH, Model no. DR6000-T
Dissolution	Electro lab
Circulating pump	Millipore Billerica, MA01821
Silverson	Silverson L5 MA
Viscometer	Brookfield DV2T

3.4 Methods

3.4.1 Development of Analytical Method

Based on literature search and using the existing analytical technique of Atorvastatin, a method has been developed and validated for analyzing the ATV/EZE combination. This section details a straightforward, efficient, and precise HPLC approach that was devised for determining the amount of Ezetimibe and Atorvastatin in a combined dosage form. The validity of this method adhered to ICH Q2(R1) guidelines through evaluation of its specificity, linearity, precision, accuracy, limit of detection, limit of quantification, as well as robustness [64].

According to the method in literature, mobile phase consisting of a 0.1 M ammonium acetate solution and acetonitrile (2:3) adjusted to pH 6.0 was used. Both drugs were detected at a wavelength of 250 nm. The pump flow rate was 1.0 ml/min, and the injection volume used was 20 $\boldsymbol{\mu l}$. Using a column Hypersil ODS C18 column (4.6 mm × 250 mm, 5 $\boldsymbol{\mu l}$) for analysis. Samples were filtered, diluted with methanol then analyzed using HPLC [2], [6]. The method in literature neither achieved detection of both of the active peaks nor met to the suitability requirements of an analysis method.

The method has been modified according to the following conditions:

Several columns have been used in the development process, until a complete separation with the desired acceptance criteria of the suitability parameters was achieved using a YMC C18 column (4.6 mm × 250 mm, 5 $\boldsymbol{\mu l}$). The supernatants were filtered and diluted with methanol at first, then diluted with the mobile phase (0.1 M ammonium acetate solution and acetonitrile (2:3) adjusted to pH 6.0) The detection wavelength for both drugs was 250 nm, the pump flow rate kept at 1.0 ml/min and the injection volumes were 20 $\boldsymbol{\mu l}$.

3.4.2 Determination of Standard, Sample, and Placebo Preparation:

3.4.2.1 Standard solution Preparation:

100 mg of ATV and 100 mg EZE were weighed into 50.0 ml volumetric flask, 30 ml of methanol were added, shaken well until complete dissolution, then the volume was completed to the mark with methanol. Then 10 ml of the solution were transferred to a 100 ml volumetric flask and diluted with mobile phase, shaken well and then the volume was completed to the mark. (ATV concentration=0.2 mg/ml, EZE concentration=0.2 mg/ml).

3.4.2.2 Sample solution Preparation:

200 mg of actives (100 mg ATV+ 100 mg EZE) were added to 5 ml of the excipients (9% oleic acid, 38% PEG, 38% Tween 80, 15% water), 2 ml were transferred to a 50 ml volumetric flask, 50 ml of Methanol was added, shaken well and the volume was completed with methanol. Then 25 ml of the solution were transferred to a 100 m volumetric flask, 100 ml of mobile phase was added, shaken well until complete dissolution (ATV concentration=0.2 mg/ml, EZE concentration=0.2 mg/ml).

3.4.2.3 Placebo solution Preparation:

The placebo solution was prepared by mixing 4000 mg of all the excipients (9% oleic acid, 38% PEG, 38% Tween 80, 15% water) in 100 volumetric flask and completing the volume with methanol, then 10 ml were transferred into 100 ml volumetric flask, and the volume was completed with mobile phase.

3.4.3 Preparing the Analytical Method Validation Protocol

The validation study was conducted according to the ICH guidelines Q2(R1 and R2). The following parameters were tested: Linearity and Range, System Precision, Method Precision, Accuracy, Specificity (Forced Degradation, Placebo Interference), Robustness and Ruggedness.

Preparations and HPLC Conditions

3.4.2.1 Mobile phase:

The mobile phase was prepared by mixing (2:3 v/v) of 0.1 M ammonium acetate solution and acetonitrile, the pH was adjusted by H_3PO_4 to 6.0 and the solution was filtered using vacuum pump.

3.4.2.2 Nominal Standard Solution preparation:

100 mg of Atorvastatin and 100 mg Ezetimibe were weighed accurately into 50.0 ml volumetric flask, 30 ml of methanol were added, accompanied with well shaking until dissolved, then the volume was completed by methanol. Then 10 ml of the solution were transferred and in a 100 ml volumetric flask, diluted with the mobile phase, shaken well and the volume was completed with the mobile phase. (ATV concentration=0.2 mg/ml, EZE concentration=0.2 mg/ml).

3.4.2.3 Nominal Sample Solution preparation:

Exact quantities of the two active ingredients (100 mg ATV+ 100 mg EZE) were added to 5 ml of the excipients (8% oleic acid+ 38% PEG400 + 38% Tween 80, 15% water), 2 ml of the previous mixture were transferred to a 50 ml volumetric flask, 50 ml of Methanol were added, shaken well then, the volume was completed with methanol. Then 25 ml of the solution were transferred to a 100 m volumetric flask, 100 ml of mobile phase were added, shaken well until completely dissolved (ATV concentration=0.2 mg/ml, EZE concentration=0.2 mg/ml).

3.4.2.4 System Suitability Requirements: [64][65]

The RSD is required to be NMT 2.0% according to the ICH Q2(R1) guidelines, Column Efficiency: NLT 2000 theoretical plates, while as the tailing factor: 0.8-2.0.

3.4.2.4.1 Forced Degradation:

This test is conducted to verify that the assay method unequivocally measures accurately and specifically the Atorvastatin/Ezetimibe in the presence of other components that may be expected to be present in the sample.

Procedure:

3.4.2.4.2 Specificity Solution:

Use the nominal sample solution prepared in section **3.4.2.3**

Different reagents were added to the Nominal Sample Solutions.

Stressed sample solutions were prepared according to Table 4.

Table 4: Solutions Preparation for Specificity Study

#	Sample Solution Conc. (mg/ml) [(1)]	Atorvastatin Sample weight (mg)	Ezetimibe sample weight	Reagent Added Stress Condition	Total Volume(ml) [3]
1	0.25mg/ml Atorvastatin + Ezetimibe	25	25	---	100
2	0.25mg/ml Atorvastatin + Ezetimibe	25	25	0.5M NaOH & Heat in Water Bath @ 80˚C, (15) min	100
3	0.25mg/ml Atorvastatin + Ezetimibe	25	25	0.5M HCl & & Heat in Water Bath @ 80˚C, (15) min	100
4	0.25mg/ml Atorvastatin + Ezetimibe	25	25	3% H2O2 &Heat	100
5	0.25mg/ml Atorvastatin + Ezetimibe	25	25	Heat in Water Bath @ 70˚C, (60) min	100
6	0.25mg/ml Atorvastatin + Ezetimibe	25	25	Under UV Light for 24hrs	100

	1: Nominal Sample Solution 2: Pipetted volume (ml) from stock sample (6.6) **3:** Volumetric flask (ml) diluted to final volume with Mph.

Sample solution is analyzed under stress conditions according to the test method of analysis for assay determination of Atorvastatin & Ezetimibe working standard. Resolution between Atorvastatin and Ezetimibe should be NLT 3.0, while as, placebo Interference NMT 2.0% [65], [66].

Placebo Interference:

This test aims at clarifying that the placebo components won't have an excessive impact on the results.

For the placebo Preparation: an emulsion was prepared according to the formulation procedure without the addition of active ingredients.

Standard Solution Preparation: a nominal standard solution was prepared for the assay test as in section 3.4.2.3

Nominal Placebo Preparation: using the placebo solution prepared previously in section **3.4.1.3**

Determine the interference of placebo using the following formula: [14]

$$Interferenc\ \% = 100 * \frac{AP}{ASt} \quad \text{..... (3)}$$

Where:

A_P: is the absorbance of the placebo

A_{St}: is the absorbance of the standard

For the results to be within the limits, Placebo interference should be kept to a maximum of 2%.

3.4.2.4.3 Robustness:

The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate changes in method parameters, and it indicates its dependability in routine use [65], [66].

Procedure:

System Suitability Solution: Use the nominal standard solution.

Variation of Method Parameters:

- **Column Oven Temperature:** ±5°C.
- **Flow Rate**: Variation of the flow rate to 0.8 ml/min and 1.2 ml/min instead of 1.0ml/min.
- **Detection Wavelength**: Variation of Detection wavelength to 252 nm and 248 nm instead of 250 nm.

Inject the nominal standard solution (prepared in point 3.4.2.2) into the Liquid Chromatograph six times and analyze it.

For the results to be within the limits, the RSD of 6 replicate injections of Atorvastatin & Ezetimibe peak area should be NMT 2.0 %, Column Efficiency: NLT 1000 Theoretical plates, and the asymmetry of the Atorvastatin & Ezetimibe peak should be NMT 0.8-2.0. [65][66]

3.4.2.4.4 Ruggedness (Intermediate Precision):

Ruggedness, also known as Intermediate Precision, is the degree of repeatability of test results when the same samples are analyzed under different settings, such as different analysts, instruments, or days.

Sample Solution: the nominal sample solution was prepared as in sections **3.4.2.3**

This test was conducted by analysis of the nominal sample solution by different analysts and on different days (matrix design). Inject the sample solution at nominal concentration using Chromatographic HPLC [65], [66].

For the results to be within the limits, the RSD for the replicate readings should be NMT 2.0%, the absolute variation should be less than 2.0%, and all System suitability results within the limit [65], [66].

3.4.2.4.5 Accuracy:

Objective: To verify that **Atorvastatin and Ezetimibe** in the emulsion are close to the true value, the accuracy of an analytical procedure measures the closeness of agreement between the value, the accuracy which is accepted reference value and value found.

It's measured as the percent of analyte recovered by assay, by spiking samples in a blind study. Accuracy is evaluated by analyzing a synthetic mixture (placebo) spiked with a known quantity of **Atorvastatin and Ezetimibe** [64]**.**

To document accuracy a minimum of nine determinations over a minimum of three concentration levels covering the specific range (for example, three concentrations, three replicates for each) were collected. It's performed at (50%-150 %) for the related test of label claim.

At each level studied, replicate samples are evaluated. The RSD of the replicate will provide the analysis variation or how the precision of the test method is. The mean of the replicate expressed as % of the label claim indicates how the accuracy of the test method.

To perform the test, the required stock placebo volume was placed into the analysis volume and the known amount of stock standard was added by volume to the analysis flask according to Table 5:

Table 5: Accuracy preparation

Conc. %	Conc. of Atorvastatin WS (mg/ml)	Conc. of Ezetimibe WS (mg/ml)	Conc. of Placebo (mg/ml)	Volumetric flask Final Volume (ml)
50%	0.125	0.125	1.25	100
100%	0.250	0.250	1.25	100
150%	0.375	0.375	1.25	100

3.4.2.4.6 Precision:

This test is conducted to demonstrate that the analytical method is capable of yielding closeness of data values between a series of measurements obtained from analysis of the same sample.

(a) System precision:

- **Procedure:** six replicates of standard preparation as per methodology. The relative standard deviation for the area of peaks of Atorvastatin and Ezetimibe is calculated
- **Acceptance criteria:** Relative standard deviation for peak areas of Atorvastatin and Ezetimibe should not be more than 2.0%.

(b) Method precision:

Procedure: Inject six different preparations of the same sample as per methodology. Calculate the relative standard deviation of the assay value of six different preparations and the results shall be tabulated.

3.4.2.4.7 Linearity and Range:

To conduct this test, each injection was run for five concentrations as given in Table 16 as per methodology.

Preparation: 25 mg Atorvastatin & 25 mg Ezetimibe were weighed in a 10 ml volumetric flask then the volume was taken according to Table 6 below.

Table 6: Linearity

Linearity level (%)	Standard Stock solution (ml)	Final volume (ml)	Area of Ezetimibe	Area of Atorvastatin
50	1	20		
75	1.5	20		
100	1	10		
125	2.5	10		
150	3	10		
Correlation coefficient				
% Intercept				

For the results to be accepted, regression line equation, the Correlation coefficient is NLT 0.995 and % intercept @ Target conc.: NMT 5% [66].

3.4.4 Preparation method for solubility tests

The main criteria for developing a SMEDDS is the screening of ingredients. First of all, the solubility of the actives is screened in oils, surfactants (SAA), and co-surfactants (Co-SAA). The efficiency of the SMEDDS to maintain a solubilized form of the drug is mainly affected by the solubility of the drug in the oily phase.

3.4.4.1 Evaluation of ATV and EZE solubility

The solubility of ATR and EZE in various excipients were examined in the following manner: (2 ml) of each of the chosen excipients were added to a screw-cap tube with excess quantities (200 mg) of both ATR and EZE. Using a vortex mixer, the mixtures were capped and mixed for 5 min. In a shaking water bath, the mixtures were agitated at 150 rpm at 40 ◦C for 72 h in order to reach equilibrium. When reaching equilibrium, each tube was centrifuged for 10 min at 5000 rpm. The precipitates will be filtered and diluted with methanol then analyzed with a validated HPLC-UV method. The detection wavelength for both drugs is 250 nm [11].

3.4.4.2 Screening of surfactants

To emulsify the chosen oil phase, various surfactants were investigated [12]. In brief, in order to achieve homogenization, equal amounts of the selected oil and surfactant were mixed and heated to 50 ◦C [14]. From the previously prepared mixtures, appropriate volume was diluted with distilled water in a glass stoppered flask. The emulsions were then visually monitored for turbidity.

3.4.4.3 Screening of co-surfactants

After making up the mind with the chosen oily phase and surfactant, varying emulsification efficiency co-surfactants were screened, including Propylene glycol® and Polyethylene glycol (PEG) 400.

In Brief, 200 μl of each co-surfactant, 400 μl of chosen surfactant and 600 μl of oily phase are mixed and evaluated visually for turbidity.

3.4.4.4 Analysis method for solubility tests

Stock solution: 100 mg of **Atorvastatin** and 100 mg **Ezetimibe** were weighed accurately into 50.0 ml volumetric flask, 30 ml of methanol were added, accompanied with well shaking until dissolved, then the volume was completed by methanol. Then 10 ml of the previous solution were transferred to a 100 ml volumetric flask and diluted with mobile phase, shaken well and the volume was completed with the mobile phase. (ATV concentration=0.2 mg/ml, EZE concentration=0.2 mg/ml).

Sample solutions: 200 mg of actives (100 mg ATV+ 100 mg EZE) were added to 5 ml of the excipients (8% oleic acid+ 38% PEG400 + 38% Tween 80, 15% water), 2 ml of the previous mixture were transferred to a 50 ml volumetric flask, 50 ml of Methanol were added, shaken well then, the volume was completed with the methanol. Then 25 ml of the solution were transferred to a 100 m volumetric flask, 100 ml of mobile phase were added, shaken well until completely dissolved (ATV concentration=0.2 mg/ml, EZE concentration=0.2 mg/ml).

3.4.5 Construction of pseudo ternary phase diagram

In order to study the behavior, recognize the self-emulsifying regions, and determine the optimum concentrations of components, Pseudo-ternary diagrams of oil, surfactant, co-surfactant, and water were composed using a water titration method [17].

Briefly, the surfactants were mixed with co-surfactant at fixed weight ratios of 1:1, 1:2, 2:1, 1:3, and 3:1. The ratios are selected based on increasing SAA concentration in respect of co-SAA and increasing co-SAA concentration in relation to SAA. Then mixed with oil at nine different ratios from 1: 9 to 9: 1. Mixtures in various vials were vigorously mixed by vortexing. Distilled water was then slowly added drop by drop to the mixtures while continuously vortexing, until equilibrium was reached. The visual transparency of the resulting mixture was assessed, and the quantities of water added to the oil, surfactant, and co-surfactant blend at the point of phase transition as determined through visual examination were recorded [67].

3.4.6 Preparation of ATV and EZE loaded SMEDDS Briefly

In order to prepare the surfactant mixture, determined ratios of SAA and co-SAA were mixed. Then, the oily phase was consistently mixed to obtain a clear homogeneous mixture. After that the actives were added slowly upon agitation till a transparent mixture was obtained [19] [20].

3.4.7 Characterization and evaluation tests

After preparation of the microemulsions, they were subjected to the following characterization and evaluation tests:

3.4.7.1 Viscosity measurement

All viscosity measurements were determined using a viscometer equipped with a spindle, and the measurements were taken at a specific temperature. each measurement was analyzed in quadrate, varying the rotation speed at 10, 20 50, and 100 RPM.

Viscosity measurements are required for droplet size measurements using DLS.

3.4.7.2 Refractive index measurement

The refractive index of each trial formulation for the microemulsion was measured using a refractometer (KRUSS Optronic GmbH, Model no. DR6000-T). The transparency of the microemulsion formulation trials can be confirmed through refractive index values.

3.4.7.3 Droplet size and size distribution

Dynamic light scattering technique (DLS) was used to determine the mean droplet size and polydispersity index of the selected microemulsion formulations. Using a Particle Sizer and Zeta Potential Analyzer - Nano Brook Omni (Brookhaven instruments), the measurements were carried out three times and recorded as mean value ± SD, in accordance with standard procedures.

3.4.7.4 Thermodynamic stability.

To examine the phase separation effect of temperature on SMEDDS formulations, evaluate physical stability, the formulations were subjected to thermodynamic stability tests:

- Heating–cooling cycle: Six cycles each of not less 48 h between 4 ◦C (refrigerator temperature) and 45 ◦C. The stable formulae had to undergo centrifugation testing.[2]
- Centrifugation test: The formulations showing stability in heating-cooling cycle test was centrifuged at 4,000 rpm for 45 min after dilution with aqueous. The formulations passing this test were subjected to freeze-thaw stress test. [2]
- Freeze–thaw cycle: Three freeze thaw cycles between a temperature (- 4 ◦C) and (+40 ◦C) were carried out, where the formulation was stored for not less than 24 h at each temperature. [2]

3.4.7.5 Dye miscibility test

This test is conducted to check whether the formed emulsion is O/W or W/O, If a water-soluble dye (methyl orange) is added in an o/w Micro-emulsion, it will be mixed homogeneously without

precipitation. On the other hand, if the Micro-emulsion is w/o and the dye being soluble in water, the emulsion takes up the color only in the dispersed phase and the emulsion is not uniformly colored [2], [6].

3.4.8 Filling in soft gel capsules

Soft gel capsules containing microemulsions have gained attention in the pharmaceutical industry due to their potential to enhance the solubility and bioavailability of drugs. The use of microemulsions in soft gel capsules is particularly promising for drugs with poor water solubility, as it can significantly improve their absorption and therapeutic effects. Additionally, the enhanced stability of these microemulsion-based soft gel capsules makes them an attractive option for drug delivery systems. The potential of this technology has sparked further research and development efforts aimed at harnessing the benefits of microemulsions in pharmaceutical applications.

In the process of loading microemulsion in a soft capsule, several steps are involved. These steps typically include preparing the microemulsion formulation, which involves combining the oil phase, water phase, and appropriate surfactants and co-surfactants. This is followed by homogenization to form the microemulsion, ensuring that the components are well-mixed and stable. The next step is calculating the density of microemulsion. The microemulsions were filled in size 00 embo caps, filling 500 mg of the formulas consisting of: 10 mg Ezetimibe, 40 mg Atorvastatin, 450 of the excipients.

3.4.9 Stability studies

The stability study involved storing the soft gel capsules filled with microemulsion formulations at different temperatures, specifically 30°C, 40°C, and in the refrigerator.

The results of the stability study conducted at different temperatures, including 30°C, 40°C, and refrigerated conditions, have provided valuable information on the capsules' stability under varied environmental stresses.

Droplet size distribution and Assay tests were conducted after stability of one month period to monitor any potential degradation, in addition to monitoring the visual appearance and phase separation.

3.4.10 In-vitro drug release studies.

Throughout the product's life cycle, a dissolution test is utilized to assess the rate at which a drug substance is released from the dosage form. This evaluation is crucial because the active pharmaceutical ingredients need to be in solution within the body before it can be absorbed into the bloodstream and reach the receptor site where it can exert its therapeutic effect.

Dissolution profile can be defined as a graphical representation in terms of [concentration vs. time] of complete release of API from dosage form and it's carried out at predetermined time points of the reference and test products using a validated dissolution method with the specified medium, as well as two extra media such as 0.1 N HCl or simulated gastric fluid without enzymes, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer or simulated intestinal fluid without enzyme. The aim of testing the product in these three media is to evaluate its dissolution performance within the biologically relevant pH spectrum [68].

In 1996, Moore and Flanner proposed two fit factors for comparing dissolution profiles: the difference factor (f 1) and the similarity factor (f 2). To use these fit factors accurately, a sufficient number of time points is needed to characterize the shape of the dissolution profiles [68], [69].

The f1 value assesses the percentage difference between the two dissolution profiles at each time point and indicates the relative error between them [68], [69].

$$f1 = \left(\frac{\sum_{t=1}^{n}|Rt-Tt|}{\sum_{t=1}^{n}Rt}\right) * 100 \quad \text{(4)}$$

The f2 metric is a mathematical transformation that represents the similarity in percentage dissolution between two profiles. Two dissolution profiles to be considered similar and bioequivalent, f1 should be between 0 and 15, whereas f2 should be between 50 and 100 [68], [69].

$$f2 = 50 * \log_{10}[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{n}(Rt-Tt)^2}{n}}}] \quad \text{..... (5)}$$

For drugs with low solubility, selecting the appropriate dissolution medium presents challenges. This is because many of the dissolution media specified in pharmacopoeias may not be able to completely dissolve poorly water-soluble drugs.

The characteristics of drug dissolution are primarily influenced by pH and surfactants. These factors are especially significant for drugs that can ionize within the pH range of the gastrointestinal tract. Among different approaches, utilizing a medium containing surfactant has been found to be appropriate due to the presence of substances such as bile salts and cholesterol in the gastrointestinal fluid [48].

Ezetimibe is insoluble in water, soluble in acetonitrile, freely soluble in ethanol and methanol, practically insoluble in aqueous media and the solubility of anhydrous and hydrated forms of the substance is approximately 12 µg/mL and 8 µg/mL, respectively, it has a pKa of 9.75, n-octanol:0.1 N HCl and n-octanol: pH 7 buffer partition coefficients (log P) of Ezetimibe are 4.52 and 4.51, respectively [48].

Atorvastatin is freely soluble in methanol [49], slightly soluble in ethanol (96%), very slightly soluble in water, practically insoluble in methylene chloride [2], [6], slightly soluble in distilled water, pH 7.4 phosphate buffer, acetonitrile, insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium has a pKa of 4.46 and octanol: water log P is 5.6 [48].

Also considering Atorvastatin and Ezetimibe may show rapid absorption, behavior on both stomach and intestinal regions are investigated by using mediums with three different pH's. Since Ezetimibe is not an ionizable drug, pH of the medium has no effect on solubility. Therefore, solubility of the Ezetimibe is enhanced by using surfactants [48].

The release of drugs from the soft gel of the SMEDDS were conducted using the following method:

Microemulsion formulations and Atozet brand were tested using the USP paddle apparatus II methodology. Capsules filled with SMEDDS formulations containing 40 mg of ATV and 10 mg of EZE were used for this experiment. To ensure the capsules remained fully submerged during dissolution testing, they were subjected to sinking conditions.

Regarding the dissolution process, the conditions were as follows: a volume of 900 ml of distilled water was used and the temperature of the medium during dissolution kept constant at 37◦C$\pm$ 0.5, while operated with a paddle speed set to 75 rpm. In accordance with previous research [48], predetermined time points of 5,10,20,30 and 45 minutes were selected for withdrawing suitable volumes of the sample solution according to FDA. These withdrawn samples were then filtered with a filter of a pore size of 0.45 μm before being immediately replaced by fresh dissolution medium to maintain equilibrium conditions [38], [39], [70], [71].

The mean dissolution time and dissolution efficacy has also been calculated in order to clarify the differences between local formulas and the brand, equation 4 is used in calculating DE.

$$DE\% = \frac{\int_0^t y.dt}{y100.t} * 100 \quad \text{..... (6)}$$

The dissolution efficiency (DE) is defined as the area under the dissolution curve (y) up to a certain time t and expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time [72].

$MDT = \sum ti.\Delta Mi / \sum \Delta Mi$……. (7)

$t = (ti + t\text{i+1})/2$…….....(8)

$\Delta M = (M\text{i+1-}M\text{i})$…….……...….... (9)

Where ti¯is the midpoint of the time period during which the fraction ΔM of the drug has been released from sample [72].

MDT is the arithmetic mean value of any release profiles and is used to describe the drug release rate, to describe the hindering ability of the used excipients and to compare different release profiles statistically.

Chapter 4

Results and discussion

4.1 HPLC method development

After applying the method in literature which was previously mentioned at section 3.1, the following chromatogram in Figure 10 resulted:

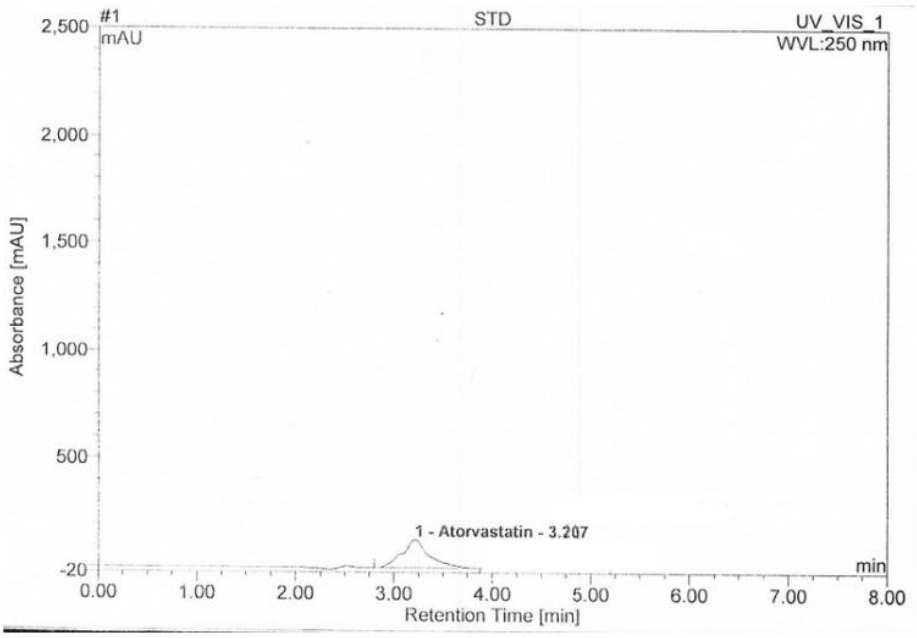


Figure 10: First attempts of analyzing ATV/EZE combinations based on literature method.

As can be seen in the chromatogram, the peak of Ezetimibe has not been detected, and the peak of Atorvastatin was not symmetrical.

After applying modifications according to the previously developed in-house method, separation of the two peaks was achieved and the method was validated and met the suitability requirements. Resulting in the following chromatogram in Figure 11:

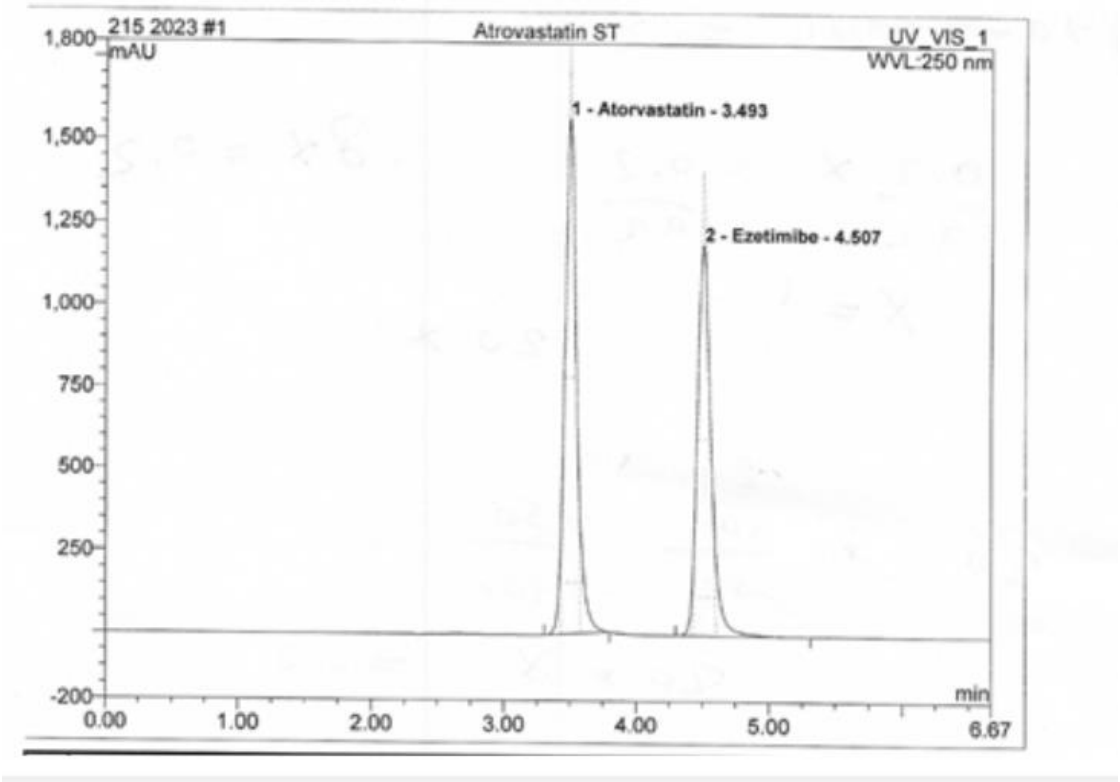


Figure 11: Chromatogram of the in-house method developed to analyze the ATV/EZE combination.

4.2 HPLC method validation

The Chromatographic Conditions of the in-house developed method where as follows:

Detection Wavelength: 250 nm, Column used: YMC C18 column (4.6 mm × 250 mm, 5 μm; Illinois, USA), Flow Rate: 1.0 ml/min, Column Temperature: Ambient, and Injection Volume: 20 µL.

Specificity:

Table 7 below clarify the results of the specificity interference test, there is no interference observed in standard and sample solution ageists of blank and placebo at the same RT.

Table 7: Specificity

Test Solution	RT	Peak Response Area
Blank	0	0
Placebo	0	0
Atorvastatin	3.12	20.989±0.63
Ezetimibe	4.82	19.504±0.302

Forced degradation:

As con be concluded from Table 8, the Degradation for Peroxide, Acid and Base which is found more than 20% for Ezetimibe at peroxide and acid which show high sensitivity.

Table 8: Forced degradation

Degradation Condition	API	Assay %	Degradation %	Acceptance
Peroxide degradation	Atorvastatin	96.7	3.3	Yes
	Ezetimibe	0.10	99.90	NO
Base degradation	Atorvastatin	90.0	10	Yes
	Ezetimibe	98.4	1.6	Yes
Acid Degradation	Atorvastatin	19.30	80.70	No
	Ezetimibe	89.80	10.20	Yes

Precision

System precision:

Table 9 below represents the results of the system precision test, the relative standard deviation for area of peaks of Atorvastatin and Ezetimibe standards was calculated.

Table 9: System precision

Replicates	Peak area of Atorvastatin	Peak area of Ezetimibe
1	70.898	70.504
2	70.314	70.064
3	70.590	70.094
4	70.775	70.348
5	70.263	70.032
6	70.328	70.074
Average	70.528	70.186
Std. Dev.	0.26	0.19
% RSD	0.37	0.27

Since the RSD for peak areas of ATV standard, and EZE isn't more than 2.0%, then the method is precise.

Accuracy

Table10 below represents the results of the accuracy test conducted, wince the results all fell within the 98-102% range, then the method is accurate.

Table 10: Accuracy as Recovery

Level No/ level in %	Actual added Amount of Atorvastatin	Amount of Atorvastatin Recovered in mg	%Recovery	% RSD
Level – 1 (50%)	10.11	9.92	98.12	3.8%
	10.45	10.30	98.56	
	10.62	10.67	100.66	
Level – 2 (100%)	20.00	20.05	100.25	4.2%
	20.10	20.06	99.74	
	20.20	20.29	100.62	
Level – 3 (150%)	30.23	30.16	99.76	1.5%
	30.50	30.72	100.97	
	30.40	30.25	99.50	
Level No/ level in %	**Actual added Amount of Ezetimibe**	**Amount of Ezetimibe Recovered in mg**	**%Recovery**	**% RSD**
Level – 1 (50%)	10.11	10.14	100.20	4.7%
	10.45	10.28	98.37	
	10.51	10.42	100.86	
Level – 2 (100%)	20.30	20.16	99.31	4.8%
	20.90	20.95	99.76	
	20.00	19.81	99.05	
Level – 3 (150%)	30.13	29.96	99.40	3.7%
	30.50	30.49	99.99	
	30.40	30.36	99.86	

Linearity and Range

Table11 below represents the linearity results, since the Correlation coefficient is NLT 0.990 for both ATV and EZE, then the method is linear. Calibration curves of Atorvastatin and ezetimibe are represented in Figures 12 and 13 with R^2 of 0.9916 and 09954, respectively.

Table 11: Linearity and range

Linearity level (%)	Standard Stock solution (ml)	Final volume (ml)	Area of Atorvastatin	Area of Ezetimibe
50	1	100	9.128	8.116
75	1.5	100	15.757	15.078
100	1	50	26.785	27.249
150	2.5	100	36.422	36.045
200	3	100	73.486	70.600
Correlation coefficient			0.991	0.9954

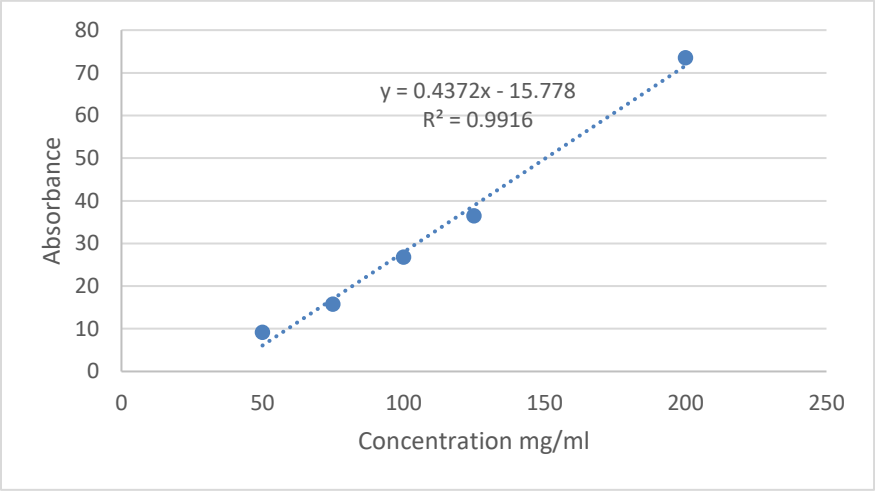


Figure 12: Standard calibration curve of Atorvastatin

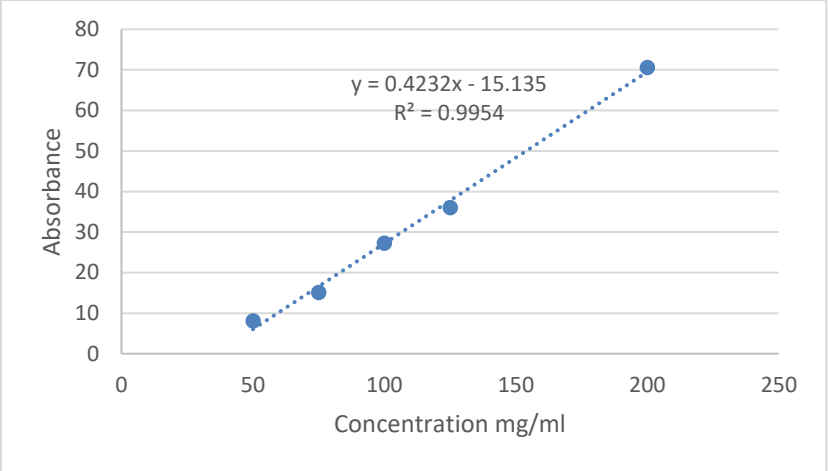


Figure 13: Standard calibration curve of Ezetimibe

Robustness:

Table 12 represents the robustness results, since RSD is less than 2% then it was concluded that the method is robust.

Table 12: Robustness flow rate Variation

Sample No.	Atorvastatin	Ezetimibe
Sample 1	71.898	71.526
Sample 2	70.056	72.54
Sample 3	71.25	73.08
Sample 4	70.985	72.52
Sample 5	71.258	71.258
Sample 6	70.396	71.985
Mean	70.973	72.151
SD	0.66	0.68
RSD (%)	0.93	0.95
Sample No.	**Atorvastatin**	**Ezetimibe**
Sample 1	68.582	67.52
Sample 2	68.147	66.521
Sample 3	69.521	65.8225
Sample 4	68.52	66.258
Sample 5	69.52	66.74
Sample 6	68.45	66.288
Mean	68.79	66.524
SD	0.58	0.57
RSD (%)	0.85	0.86

Table 13: Validation summary

Parameter	Acceptance criteria	Result	Conclusion
Specificity	Interference test: There should be no interference due to blank, placebo.	No interference observed	Confirm
	Forced degradation: degradation % from 5-20%	Degradation is within the limits	Confirm
Accuracy	Accuracy or % Recovery at each concentration should be between 80 - 120.0 The % RSD for recovery of triplicate preparation for each level should not be more than 5.0%	% RSD of ATV: Level 1 (50%) =3.8 Level 2 (100%) =4.2 Level 3 (150%) =1.5 % RSD of EZE: Level 1 (50%) =4.7 Level 2 (100%) =4.8 Level 3 (150%) =3.7	Confirm
Precision	System precision: The % Relative standard deviation of the five standard NMT 2.0 %	RSD is less than 1.0%	Confirm
Linearity and range	Correlation coefficient is NLT 0.990.	r^2 of Atorvastatin = 0.9910 $r^2$of Ezetimibe = 0.9954	Confirm
Robustness	The Relative standard deviation of the 10% variation in flow rate, and UV wavelength should Not more than 2.0 %	RSD Less than 2%	Confirm

4.2 Evaluation of ATV and EZE solubility in various oils

The solubility of the actives has been studies in various oily phases: Castor oil, Isopropyl myristate, Olive oil, Soybean oil, Ethyl oleate, Sesame oil, Oleic acid and Triacetin, as can be seen in Table 14. The saturation solubility of both EZE and ATV has been the highest in Oleic acid and in Triacetin compared to other oils.

The enhancement of ATV/EZE solubility may owe to solubilizing capacities of Triacetin for lipophilic agents, Triacetin, also known as glyceryl triacetate is a water-soluble short-chain triglyceride that is a

water miscible solvent with three hydroxy groups of glycerol. It possesses several distinctive traits as non-toxicity and the ability to form self-emulsifying formulations, making it suitable for use as both a co-solvent and an emulsification aid [73].

Oleic acid is an unsaturated fatty weak acid with an HLB value of 1.0, and it's a long-chain carboxylic acid with p*K*a value of 3.8 and log *P* (*n*-octanol/water) values of 2.29 and 1.8 [74]. The high solubility of ATV/EZE in oleic acid may owe to the complexation resulting between the carboxylic groups of oleic acid and the actives [74].

It has been reported that the use of castor oil as the oil phase in any system did not result in the formation of a microemulsion [2]. This could be due to differences in molecular volume between castor oil and the other oils used. Castor oil is primarily composed of triglycerides of ricinoleic acid, which has a larger molecular weight (933.61) compared to ethyl oleate with a straight chain structure and a molecular weight of 310.51, The three-chain (triglyceride) structure provides long chain lengths contributing to the high molecular volume of castor oil making its incorporation into microemulsion droplets challenging.

Table 14: Saturation solubility of actives in various oils

Sample	Solubility of ATV (mg / ml)	Solubility of EZE(mg / ml)
ISPM	0.273±0.23	0.023 ±0.24
Sesame oil	0.027±0.30	0.015±1.48
Ethyl Oleate	0.033±1.39	0.034±0.12
Olive Oil	0.034±0.37	0.008±0.36
Soybean Oil	0.146±0.200	0.011±1.74
Castor Oil	0.055±0.14	0.005±12.63
Oleic acid	0.673±0.031	0.062±5.610
Triacetin	8.07±2.37	7.39±0.30

4.3 Evaluation of ATV and EZE solubility in various surfactants

The solubility has also been studies in all of the excipients used including surfactants and co-surfactants, Table 15 below shows the differences in solubility. Surfactants solubility was in the

order of Tween 20 > Tween 80> Kolliphor RH 40.

Surfactant mixtures with a high HLB value (such as tween 20 and tween 80 with HLB value of 16.7, and 15 respectively) demonstrate enhanced emulsification efficiency, enabling the easy and rapid dispersion of the oily phase in the aqueous phase to create extremely fine o/w emulsions. Additionally, they offer the advantage of exhibiting bioactive effects as nonionic surfactants, such as the lymphotropic properties of Tween 80 and the inhibitory effect on p-gp and CYP enzymes demonstrated by Kolliphor RH 40 [75].

In addition to that, the structure of Kolliphor RH40 features a branched alkyl chain, unlike the linear structure of Tween 80. According to Borhade et al., it has been noted that the branched alkyl structure of the surfactant is more effective for solubility and microemulsion formation [73], [76], [77].

Table 15: Saturation solubility of actives in various surfactants and co-surfactants

Sample	Function	Solubility of ATS (mg / ml)	Solubility of EZE (mg / ml)
PG	Co-surfactant	0.8991 ±0.06	0.0025±2.32
PEG 400	Co-surfactant	0.8567±0.16	0.1037±0.36
Tween 80	Surfactant	3.021±0.8	2.473±0.17
Tween 20	Surfactant	5.844±1.17	4.932±0.37
Kolliphor RH 40	Surfactant	0.5413 ±0.26	0.3822±0.43

Figure 14 below demonstrates the variability in solubility of the ATV/EZE in all the excipients used for formulating microemulsions.

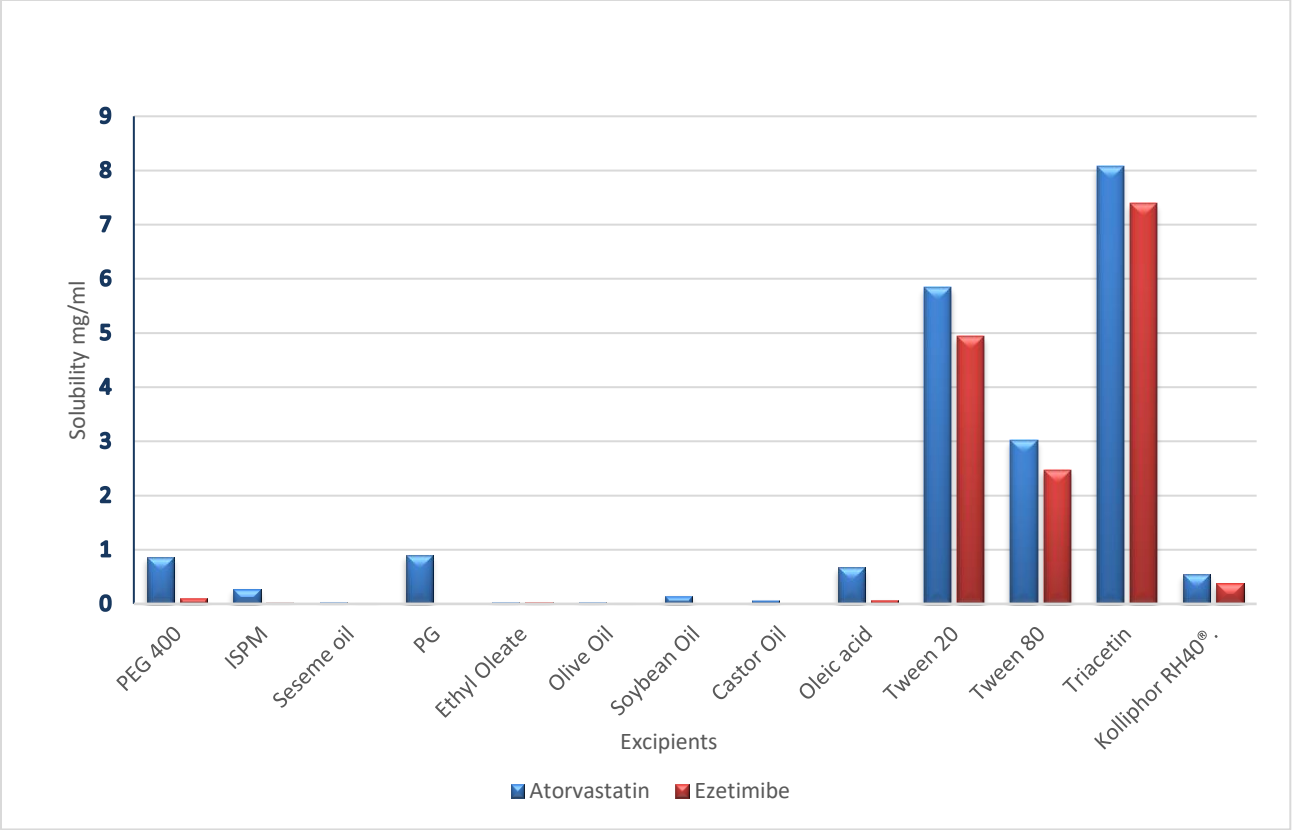


Figure 14: Saturation solubility of actives in all excipients

4.4 Formulation trials using pseudo ternary phase diagrams

A pseudo-ternary phase diagram can be a valuable tool for creating an appropriate SMEDDS composition, consisting of drug, oil, surfactant, co-surfactant, and water. Typically, this diagram reveals three phases: microemulsion, liquid crystal, and coarse emulsion. The focus is mainly on the microemulsion region when formulating SMEDDS. A wide microemulsion region allows for greater flexibility in determining the ideal dosage composition. Microemulsions are recognized by their transparent and clear appearance.

In this phase, different experiments were conducted to create formulations, using phase titration technique along with pseudo ternary phase diagrams, according to Table 16 below, which explains the method applied in preparation along with the percentages of excipients.

The endpoint for each formulation was identified and the volume percentage of the components (oil, surfactant/co-surfactant, and water) in these micro emulsion trials was calculated. These values were then plotted on triangular coordinates to develop pseudo-ternary phase diagrams.

Ratios of Oil: (surf. + co-surf.) ranged from 1:9 to 9:1, while as Surfactant: Co-surfactant ratios used were: 1:1, 1:2, 2:1, 1:3, and 3:1. The percentages of water ranged from 5%-70%.

Table 16: Micro emulsion formulation ratios using titration method and pseudo-ternary diagrams.

Oil: (surf. + co-surf.) ratio	Surfactant: Co-surfactant ratio	% Water
1:9	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
2:1	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
1:4	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
1:9	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
2:8	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
3:7	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
4:6	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
5:5	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
6:4	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
7:3	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
8:2	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%
9:1	1:1, 1:2, 2:1, 1:3, 3:1	5% -70%

Table 17 below, clarifies the percentages of excipients added to all the possibilities of systems created throughout the whole formulation process. All Tables constructed are attached in appendix B (Table B1 – Table B36).

Table 17: Preparation method of pseudo-ternary diagram

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**														
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**														
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**														
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**														
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**														
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**														
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**														
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**														
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**														

4.4.1 Pseudo-ternary phase diagrams

As can be seen in Figures 15-19, pseudo-ternary phase diagrams were constructed – with the absence of the active materials- for all systems, employing: Oleic acid, Triacetin as the oily phases, Tween 80, Tween 20, Kolliphor RH40 as surfactants, and PEG 400, PG as the co-surfactants, representing the areas of microemulsions. The rest of the systems are represented in appendix C.

As can concluded from the Figures (15-19) below, Triacetin exhibited greater microemulsion areas than those constructed by oleic acid, which might be attributed to the fact that Triacetin has three ester groups that impart some hydrophilic nature to the formula resulting in an increased solubility and emulsification capacity. Also, the low HLB value of oleic acid may have contributed to the fact that it has lower solubilization effect and low emulsification capabilities [74].

Also, a broader micro-emulsion region was achieved with a higher ratio of surfactant. This finding aligns with literature, where a wider microemulsion region was obtained using higher ratio of surfactant. The increased concentration of surfactant at the interface potentially enhances its adsorption and reduces interfacial tension, leading to micro-emulsion formation [76].

Also, Formulations with higher oil concentration (higher than 25%) exhibited phase separation, possibly due to the coalescence of oil droplets as previously explained [76].

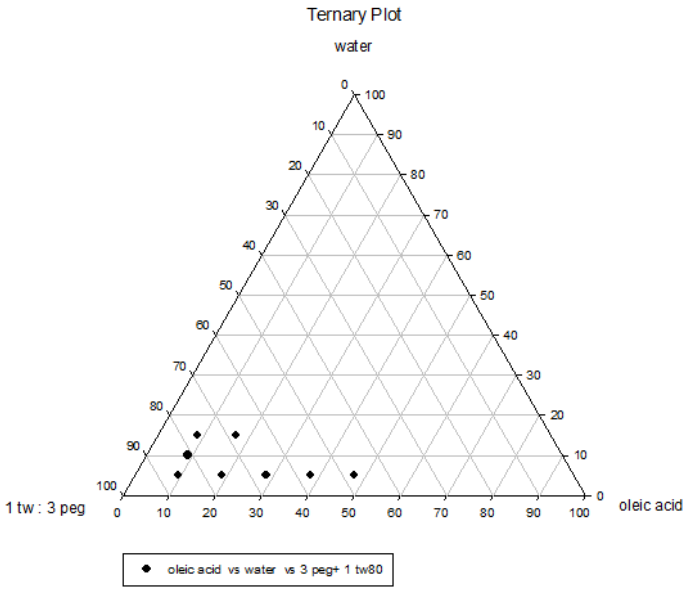


Figure 15: Pseudo ternary phase diagram of Oleic acid+ water+ PEG 400/TW 80 (3:1)

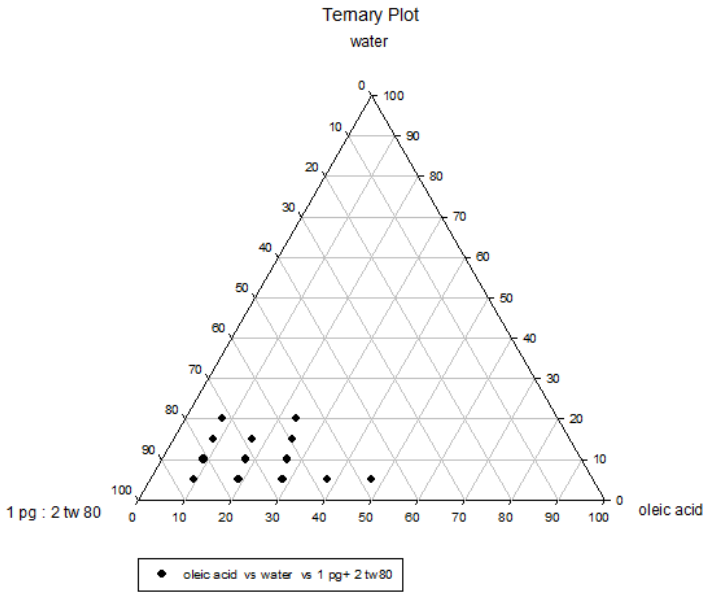


Figure 16: Pseudo ternary phase diagram of Oleic acid+ water+ PG/ TW 80 (1:2)

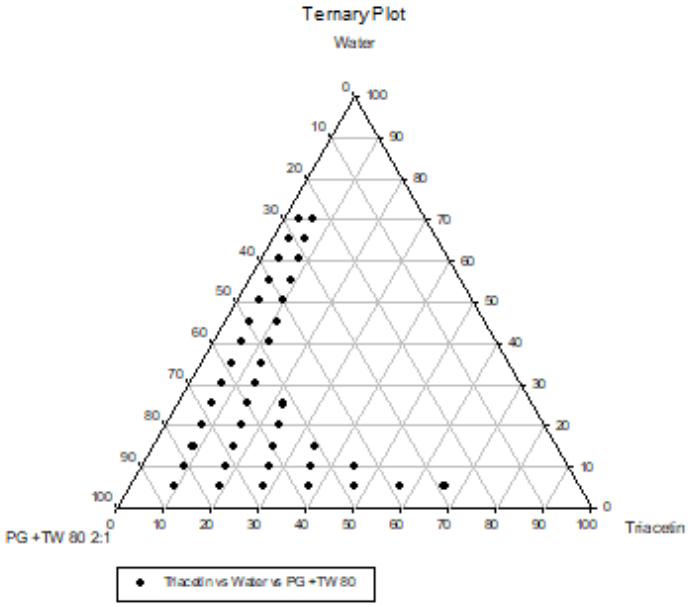


Figure 17: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (2:1)

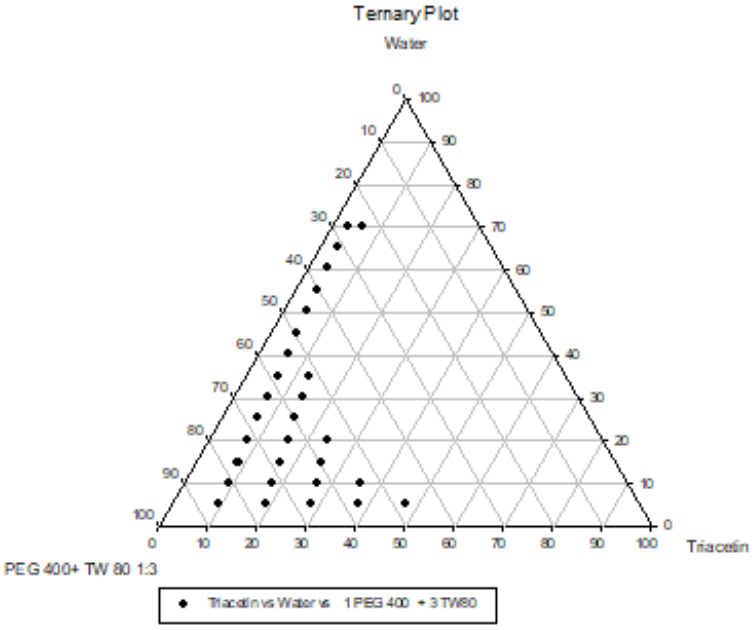


Figure 18: Pseudo ternary phase diagram of Triacetin+ water+ PEG400 /TW 80 (1:3)

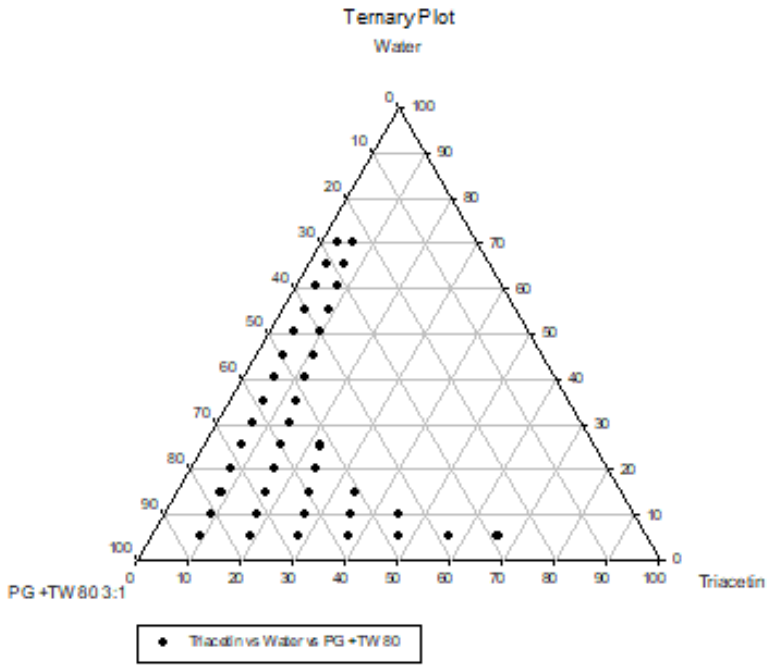


Figure 19: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1)

4.5 Type and composition of the selected micro emulsionformulations trials

Upon constructing all pseudo ternary phase diagrams of all possible combinations of excipients, 17 formulas having oleic acid as oily phase, and 28 formulas having Triacetin as the oily phase, -which had clear monophasic configuration- has been selected for further studying.

The selected formulation trials of micro emulsion illustrated in Table 18 were subjected to physical properties testing including viscosity, refractive index, droplet size and polydispersity index.

In addition to testing the type of the microemulsion using Sudan III dye according to the method explained in section (**3.3.6.5**).

Table 18: Selected formulation trials of micro emulsion with (v/v%) using ternary diagram Tables.

ME #	Composition	Ratio Oil: surf. /co-surf.	Ratio surf. /co-surf.	Water %	Oil %	Surf. %	co-surf. %	Type ofME
#T1	Oleic acid: T80/ PG	1:9	1:1	15	8.50	38.24	38.24	O/W
#T2	Oleic acid: T80/ PG	1:9	3:1	15	8.50	57.36	19.12	O/W
#T3	Oleic acid: T80/ PG	3:7	3:1	15	25.45	44.66	14.89	O/W
#T4	Oleic acid: T80/ PEG	1:9	1:1	15	8.50	38.24	38.24	O/W
#T5	Oleic acid: T80/ PEG	1:9	1:2	10	8.99	26.99	53.98	O/W
#T6	Oleic acid: T20/ PG	1:9	1:1	20	7.99	35.95	35.95	O/W
#T7	Oleic acid: T20/ PG	1:9	1:3	20	4.97	17.97	53.93	O/W
#T8	Oleic acid: T20/ PG	2:8	1:1	10	17.99	35.98	35.98	O/W
#T9	Oleic acid: T20/ PG	1:9	1:2	20	7.98	23.97	47.94	O/W
#T10	Oleic acid: T20/ PG	3:7	2:1	25	23.93	37.39	18.66	O/W
#T11	Oleic acid: T20/ PEG 400	1:9	1:1	25	7.48	33.66	33.66	O/W
#T12	Oleic acid: Koll RH 40/ PG	1:9	1:1	15	8.49	38.23	38.23	O/W
#T13	Oleic acid: Koll RH 40/ PEG 400	1:9	1:1	15	8.497	38.23	38.23	O/W
#T14	Oleic acid: Koll RH 40/ PEG 400	3:7	1:1	15	25.44	29.77	29.77	O/W
#T15	Oleic acid: Koll RH 40/ PEG 400	1:9	3:1	25	7.48	50.49	16.83.	O/W
#T16	Oleic acid: Koll RH 40/ PEG 400	1:9	2:1	15	8.49	51	25.49	O/W
#T17	Oleic acid: Koll RH 40/ PEG 400	2:8	1:1	20	15.97	31.95	31.95	O/W
#T18	Triacetin: Tw 80/PG	1:9	1:1	70	2.97	13.36	13.36	O/W
#T19	Triacetin: Tw 80/PG	2:8	1:1	70	5.94	11.88	11.88	O/W
#T20	Triacetin: Tw 80/PG	1:9	1:2	70	2.97	6.675	20.67	O/W
#T21	Triacetin: Tw 80/PG	1:9	2:1	70	2.97	8.9	17.8	O/W
#T22	Triacetin: Tw 80/PG	2:8	2:1	25	14.97	39.9	19.9	O/W
#T23	Triacetin: Tw 80/PG	1:9	1:3	70	2.97	6.68	20	O/W
#T24	Triacetin: Tw 80/PG	1:9	3:1	70	2.97	20	6.68	O/W
#T25	Triacetin: Tw 80/PG	2:8	3:1	30	13.99	41.97	13.99	O/W
#T26	Triacetin: Tw 80/PG	3:7	3:1	15	25.45	44.66	14.89	O/W
#T27	Triacetin: Tw 80 / PEG 400	1:9	1:1	70	2.96	13.35	13.35	O/W
#T28	Triacetin: Tw 80 / PEG 400	2:8	1:1	25	14.96	29.92	29.92	O/W
#T29	Triacetin: Tw 80 / PEG 400	1:9	1:2	70	2.96	8.90	17.81	O/W
#T30	Triacetin: Tw 80 / PEG 400	1:9	2:1	70	2.96	17.81	8.90	O/W
#T31	Triacetin: Tw 80 / PEG 400	2:8	2:1	25	14.96	39.89	19.94	O/W

#T32	Triacetin: Tw 80 / PEG 400	1:9	1:3	70	2.96	6.67	20.03	O/W
#T33	Triacetin: Tw 80 / PEG 400	2:8	3:1	30	13.99	41.97	13.99	O/W
#T34	Triacetin: Tw 20 / PG	2:8	1:1	40	11.95	23.9	23.9	O/W
#T35	Triacetin: Tw 20 / PEG 400	2:8	1:1	25	14.96	29.92	29.92	O/W
#T36	Triacetin: Tw 80/PG	1:9	3:1	40	5.97	40.33	13.44	O/W
#T37	Triacetin: Tw 80/PG	1:9	1:3	35	6.49	14.62	43.86	O/W
#T38	Triacetin: Tw 80/PG	1:9	1:2	30	6.99	20.98	41.97	O/W
#T39	Triacetin: Tw 80/PG	1:9	2:1	25	7.48	44.88	22.44	O/W
#T40	Triacetin: Tw 80/PG	2:8	1:2	40	11.95	15.93	31.86	O/W
#T41	Triacetin: Tw 80/PG	2:8	1:3	20	15.98	15.98	47.94	O/W
#T42	Triacetin: Tw 80 / PEG 400	1:9	1:3	50	4.97	11.19	33.57	O/W
#T43	Triacetin: Tw 80 / PEG 400	2:8	3:1	20	15.98	47.94	15.98	O/W
#T44	Triacetin: Tw 80/PG	3:7	1:1	15	25.45	29.77	29.77	O/W
#45	Triacetin: Tw 80/PG	2:8	1:1	35	12.99	25.99	25.99	O/W

Table 18 above presents the composition, ratios, and types of microemulsion formulations. After applying the dye test (Sudan III), All the formulations appeared to have O/W configuration with a clear monophasic visual appearance. These formulations were chosen for evaluating their physical characteristics to determine which ones fall within the accepted criteria of a microemulsion, droplet size (10 – 100 nm), polydispersity index(0.1-0.7), refractive index (1.30 – 1.45), and viscosity (10 – 400 cP) [2].

4.6 Summary of physical properties of the selected micro emulsionformulation trials.

Table 19 below shows the summary of physical characteristics for the 45 selected micro emulsion formulation trials.

Table 19: Summary of physical properties for the selected micro emulsion formulationtrials.

ME#	Composition	Ratio Oil: surf. /co-surf.	Ratio Surf. /co-surf.	Visual appearance	Viscosity(cp)	RI	Droplet size (nm)	Polydispersity
#T1	Oleic acid: T80/ PG	1:9	1:1	Monophasic	323.7 ± 1.05	1.4361	0.75±0.05	0.37±0.006
#T2	Oleic acid: T80/ PG	3:7	3:1	Monophasic	440.5 ± 0.80	1.4426	0.78±0.11	0.33±0.05
#T3	Oleic acid: T20/ PG	1:9	1:1	Monophasic	411.8 ± 1.32	1.4428	3.57±0.36	0.35±0.02
#T4	Oleic acid: T20/ PG	1:9	1:3	Monophasic	260 ± 0.54	1.4362	2.03±0.77	0.27±0.09
#T5	Oleic acid: T20/ PG	2:8	1:1	Monophasic	148.5 ± 1.42	1.4361	3.73±0.22	0.24±0.06
#T6	Oleic acid: T20/ PG	1:9	1:2	Monophasic	184.5 ± 1.91	1.4306	3.12±1.98	0.22±0.08
#T7	Oleic acid: T20/ PG	1:9	3:1	Monophasic	111.5 ± 1.9	1.4336	3.16±0.05	0.2±0.03
#T8	Oleic acid: T20/ PG	3:7	2:1	Monophasic	158.5 ± 0.44	1.4406	3.27±0.14	0.3±0.02
#T9	Oleic acid: T20/ PEG 400	1:9	1:1	Monophasic	115.3 ± 0.85	1.4259	1.89±0.03	0.27±0.03
#T10	Oleic acid: Koll RH 40/ PG	1:9	1:1	Monophasic	456.5 ± 1.70	1.4326	2.57±0.04	0.36±0.01
#T11	Oleic acid: Koll RH 40/ PG	2:8	1:1	Monophasic	249.3 ± 2.12	1.4344	1.84±0.07	0.29±0.03
#T12	Oleic acid: Koll RH 40/PEG 400	1:9	1:1	Monophasic	300.3 ± 0.35	1.4329	73.12±8.64	1.76±0.92
#T13	Oleic acid: Koll RH 40/PEG 400	2:8	1:1	Monophasic	261.5 ± 1.35	1.4364	3.32±0.62	0.28±0.08
#T14	Oleic acid: Koll RH 40/PEG 400	3:7	1:1	Monophasic	233.4±0.51	1.4321	4.58±0.31	0.26±0.23
#T15	Oleic acid: Koll RH 40/PEG 400	1:9	3:1	Monophasic	212.4±1.56	1.4402	2.33±0.45	0.16±0.3
#T16	Oleic acid: Koll RH 40/PEG 400	1:9	2:1	Monophasic	239.2±2.72	1.4321	6.73±0.13	0.24±0.12
#T17	Oleic acid: Koll RH 40/PEG 400	2:8	1:1	Monophasic	176.3±1.2	1.3723	6.22±0.44	0.47±0.18
#T18	Triacetin: Tw 80/PG	1:9	1:1	Monophasic	10.3 ± 1.37	1.3712	1.75±0.02	0.26±0.012
#T19	Triacetin: Tw80/PG	2:8	1:1	Monophasic	11.3 ± 10.01	1.3705	1.70±0	0.25±0.09
#T20	Triacetin: Tw 80/PG	1:9	1:2	Monophasic	7.23 ± 0.58	1.3702	2.33±0.01	0.21+0.01
#T21	Triacetin: Tw80/PG	1:9	2:1	Monophasic	9.14 ± 4.02	1.3733	2.86±0.03	0.22±0.003
#T22	Triacetin: Tw 80/PG	2:8	2:1	Monophasic	12.31 ± 1.03	1.4302	51.73±2.97	0.35±0.03
#T23	Triacetin: Tw 80/PG	1:9	1:3	Monophasic	6.62 ± 1.70	1.3684	3.92±0.17	0.224±0.006
#T24	Triacetin: Tw 80/PG	1:9	3:1	Monophasic	18.3 ± 0.50	1.3722	1.28±0.28	0.143±0.049
#T25	Triacetin: Tw 80/PG	2:8	3:1	Monophasic	309.5 ± 1.14	1.4212	0.60±0.03	0.268±0.021
#T26	Triacetin: Tw 80/PG	3:7	3:1	Monophasic	244.4 ± 0.08	1.4401	16.21±2.03	0.068±0.050
#T27	Triacetin: Tw80/PEG 400	1:9	1:1	Monophasic	12.72 ± 0.66	1.3709	3.20±1.52	0.107±0.076

#T28	Triacetin: Tw 80 /PEG 400	2:8	1:1	Monophasic	12.53± 0.31	1.3523	2.26± 0.72	0.37±0.042
#T29	Triacetin: Tw 80 /PEG 400	1:9	1:2	Monophasic	10.5 ± 4.04	1.3687	1.43±0.01	0.207±0.01
#T30	Triacetin: Tw 80 /PEG 400	1:9	2:1	Monophasic	12.78 ± 4.64	1.4305	1.16±0.01	0.251±0.019
#T31	Triacetin: Tw 80 /PEG 400	2:8	2:1	Monophasic	310 ± 9.58	1.3708	74.15±1.68	0.337±0.022
#T32	Triacetin: Tw 80 /PEG 400	1:9	1:3	Monophasic	8.16 ± 6.23	1.3272	1±0.02	0.226±0.005
#T33	Triacetin: Tw 80 /PEG 400	2:8	3:1	Monophasic	83.7 ± 5.57	1.4193	1.92±0.32	0.245±0.042
#T34	Triacetin: Tw20 / PG	2:8	1:1	Monophasic	63.3 ± 0.67	1.3990	1.21±0.06	0.265±0.033
#T35	Triacetin: Tw 20 /PEG 400	2:8	1:1	Monophasic	65.8 ± 0.32	1.4252	10.55±2.72	0.173±0.035
#T36	Triacetin: Tw 80/PG	1:9	3:1	Monophasic	120 ± 1.17	1.4078	5.80±3.30	0.198±0.068
#T37	Triacetin: Tw 80/PG	1:9	1:3	Monophasic	20.3 ± 5.9	1.4010	1.57±0.05	0.248±0.005
#T38	Triacetin: Tw 80/PG	1:9	1:2	Monophasic	41.4 ± 0.63	1.4128	1.19±0.11	0.207±0.072
#T39	Triacetin: Tw 80/PG	1:9	2:1	Monophasic	384.2 ± 0.55	1.4250	3.54±4.60	13.78±23.125
#T40	Triacetin: Tw 80/PG	2:8	1:2	Monophasic	31 ± 9.12	1.4001	1.10±0.04	0.151±0.030
#T41	Triacetin: Tw 80/PG	2:8	1:3	Monophasic	50.2 ± 1.00	1.4194	1.62±0.03	0.298±0.020
#T42	Triacetin: Tw 80 /PEG 400	1:9	1:3	Monophasic	20.5 ± 7.58	1.4046	3.78±0.06	0.236±0.004
#T43	Triacetin: Tw 80 /PEG 400	2:8	3:1	Monophasic	330.2 ± 18.91	1.4379	1.61±0.16	0.306±0.063
#T44	Triacetin: Tw 80/PG	3:7	1:1	Monophasic	78.6 ± 0.1	1.4294	1.99±1.05	0.206±0.173
#T45	Triacetin: Tw 80/PG	2:8	1:1	Monophasic	80.5 ± 0.17	1.4101	2.27±2.17	0.113±0.066

Viscosity measurements were established using Brookfield viscometer and ranged between (6.62-440).

The refractive index values prove the transparency and the isotropic nature of the micro emulsion formulation trials. Which were almost the same as the Refractive index of water which equals 1.3316.

The results from the viscosity and the refractive index were used to determine the droplet size and the polydispersity index using the DLS device.

Microemulsions with a small average droplet size have the potential to enhance drug absorption and bioavailability, as well as improve stability. The size of the droplets is influenced by various factors such as the type and quantity of surfactant, along with other additives.

The droplet size of each micro emulsion formulation trial was obtained from DLS as mentioned in section (3.3.6.4), the results ranged between (0.75 -74.15).

It was noticed that smallest droplet size was achieved at oil content of 5-10% (w/w).

And as the percentages of oil increased the micro emulsion had phase separation. It was found that the optimum percentage is around 15% (w/w). Figure 20 below illustrates the relationship observed between the oil content and the mean droplet size.

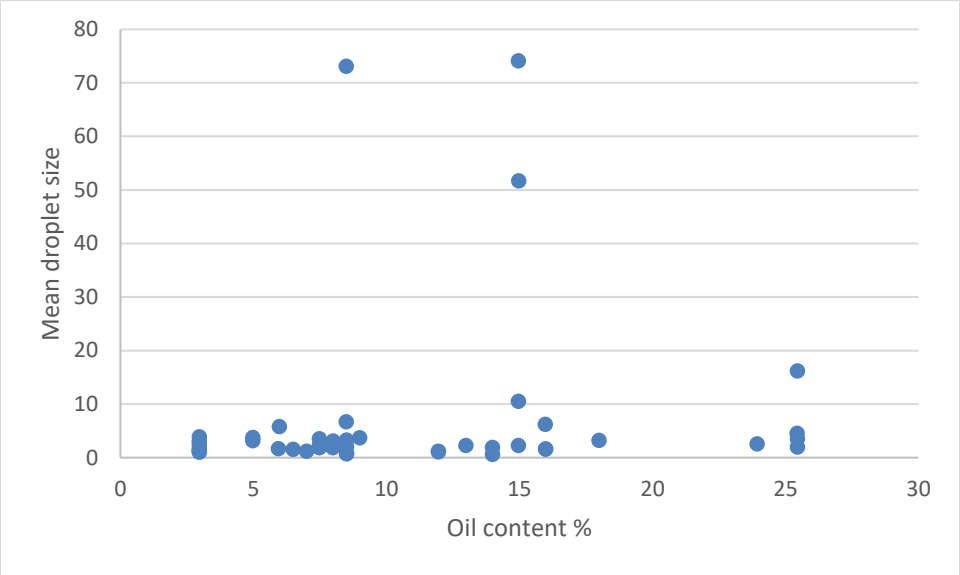


Figure 20: Effect of Oil Content on Mean Droplet Size of SMEDDS

The increase in droplet size, exceeding 25% (w/w) oil content, might be attributed to the swelling of droplets caused by higher oil content. Additionally, as the oil content increases, there is a decrease in surfactant content which allows droplets to reassemble into larger structures. This could be due to the development of multi-layered droplets through the condensation of surplus surfactant on the current droplet framework [78],[78].

In addition, Oleic acid resulted in a smaller particle size, while Triacetin led to the production of larger particles. This difference may be attributed to the lower HLB value of oleic acid (HLB=1.0)

Also, larger Particle Sizes developed when elevated concentrations of the surfactant were used in the aqueous phase, causing a higher viscosity [79].

The polydispersity index is a measure used to indicate the uniformity of particle size distribution. Referred to as the heterogeneity index, PDI is calculated from correlation data using two parameters [78]. It is a dimensionless value, where smaller values below 0.05 are typically observed in highly monodisperse standards, while larger values exceeding 0.7 suggest that the sample has a wide particle size distribution and may not be suitable for dynamic light scattering analysis.

As can been seen in Table 20, polydispersity index values ranged from 0.116 to 13.78,

The formulas that had a value less than 0.1 and greater than 0.7 were excluded. While as, the values that fell within this range were considered a homogeneous microemulsion with a narrow size distribution.

According to the results obtained, formulas 12, 22,26,31, and 35 were chosen for stability and in vitro dissolution testing as shown in Table 20.

Table 20: Formulas that met the physical accepted criteria

ME #	Composition	Ratio Oil: surf. /co-surf.	Ratio Surf. /co-surf.	Viscosity (cp)	RI	Droplet size (nm)	Polydispersity
#T12	Oleic acid: Koll RH 40/PEG 400	1:9	1:1	300.2 ± 0.35	1.433	73.12±8.64	1.76±0.92
#T22	Triacetin: Tw 80/PG	2:8	2:1	12.31 ± 1.03	1.432	51.73±2.97	0.35±0.03
#T26	Triacetin: Tw 80/PG	3:7	3:1	244.35±0.08	1.441	16.21±2.03	0.07±0.05
#T31	Triacetin: Tw 80 /PEG 400	2:8	2:1	310±9.58	1.372	74.15±1.68	0.34±0.02
#T35	Triacetin: Tw 20 /PEG 400	2:8	1:1	65.85±0.32	1.425	10.55±2.72	0.17±0.04

4.7 Solubility studies of ATV/EZE in various micro emulsion formulations

Table 21below shows the saturation solubility of ATV/EZE in the five formulas that met the acceptance criteria of the physical characteristics of micro emulsion formulations.

Table 21: Saturation solubility of ATV/EZE in various micro emulsion formulation trials.

Formula	Composition	Ratio Oil: surf. /co-surf.	Ratio surf. /co-surf.	Solubility of ATV (mg / ml)	Solubility of EZE (mg / ml)
ME#12	Oleic acid: Koll RH 40/ PEG 400	1:9	1:1	0.53 ± 0.24	0.24 ± 0.14
ME #22	Triacetin: Tw 80/PG	2:8	2:1	3.282 ± 0.34	2.60 ± 0.17
ME #26	Triacetin: Tw 80/PG	3:7	3:1	2.11 ± 0.17	2.05 ± 0.02
ME #31	Triacetin: Tw 80 / PEG 400	2:8	2:1	3.95 ± 0.35	3.04 ± 0.06
ME #35	Triacetin: Tw 20 / PEG 400	2:8	1:1	2.73 ± 0.23	2.27 ± 0.02

As seen in Figure 21 below, Formula 31 (Triacetin=14.96%, Tween 80= 39.89%, PEG 400=19.94%) had the highest solubilisation capacity among the five formulas (ATV= 3.95 mg/ml, EZE= 3.0 mg/ml), followed by formula 22 (Triacetin= 14.96%, Tween 80= 39.89%, PG=19.9%) with solubility of (ATV= 3.282 mg/ml, EZE= 2.60 mg/ml), followed by formula 35 (Triacetin= 14.96%, Tween 20= 29.92%, PEG 400= 29.92%) with solubility of (ATV= 2.73 mg/ml, EZE= 2.27 mg/ml).

According to the results obtained from the solubility and the pseudo ternary phase diagrams, it was noticed that at a ratio of Oil: surf/co-surf. 1:9, As the amount of oil decreased from 9.5% to 3%, the system remained clear and monophasic. While as, at a ratio of 2:8, as the percentage of the oily phase decreased, the system became cloudier and had phase separation.

When comparing formulas 31 and 35, which both had the same ingredients but differed in the surfactant used, and in the amount of Surf: Co-surf. Ratio, with a ratio of (2:1) for formula 31 and a ratio of (1:1) of

formula 35, which meant that a Surf: Co-surf ratio of (2:1) has better self-micro emulsification efficiency than a ratio of (1:1). In other words, increased amount of Tween 80 resulted in better solubility in addition to resulting in a more suitable droplet size of 74.15.

Tween 80 serves as an effective surfactant for both EZE and ATR due to its ability to impede P-gp efflux. Additionally, it hinders both hepatic and intestinal CYP3A4 activity, potentially improving the bioavailability of ATR [67].

Also, when comparing formulas 22 and 26 which both had same ingredients and differed in both the ratio of Oil: surf/co-surf and the ratio of Surf: Co-surf, formula 22 had an Oil: Sur/Co-Sur ratio of (2:8), meanwhile, formula 26 had a ratio of (3:7). Also, formula 22 had a Surf: Co-Surf ratio of (2:1), meanwhile, formula 26 had a ratio of (3:1).

A surfactant mixture system with a high HLB value demonstrates improved emulsification efficiency, enabling the rapid dispersion of an oily phase in an aqueous phase to create a very fine o/w emulsion. Due to the bulky polyoxyethylene groups in Tween 80 and its higher solubility in water, it has a tendency to form O/W emulsions. Which explains why microemulsions having tween 80 at high percentages up to 40% have better emulsification properties.

When Co-surfactant was mixed with surfactant in equivalent proportions (Smix 1:1), a broader microemulsion area was observed, leading to improved emulsification efficiency. This may be attributed to further reduction of the interfacial tension and increased fluidity at the interface [2].

By increasing the concentration of surfactant at ratios Smix (2:1) and (3:1), the area of the microemulsion increased compared to that at ratio Smix (1:1). Therefore, it can be concluded that an increase in SAA concentration relative to co-SAA results in an expansion of the microemulsion region [2].

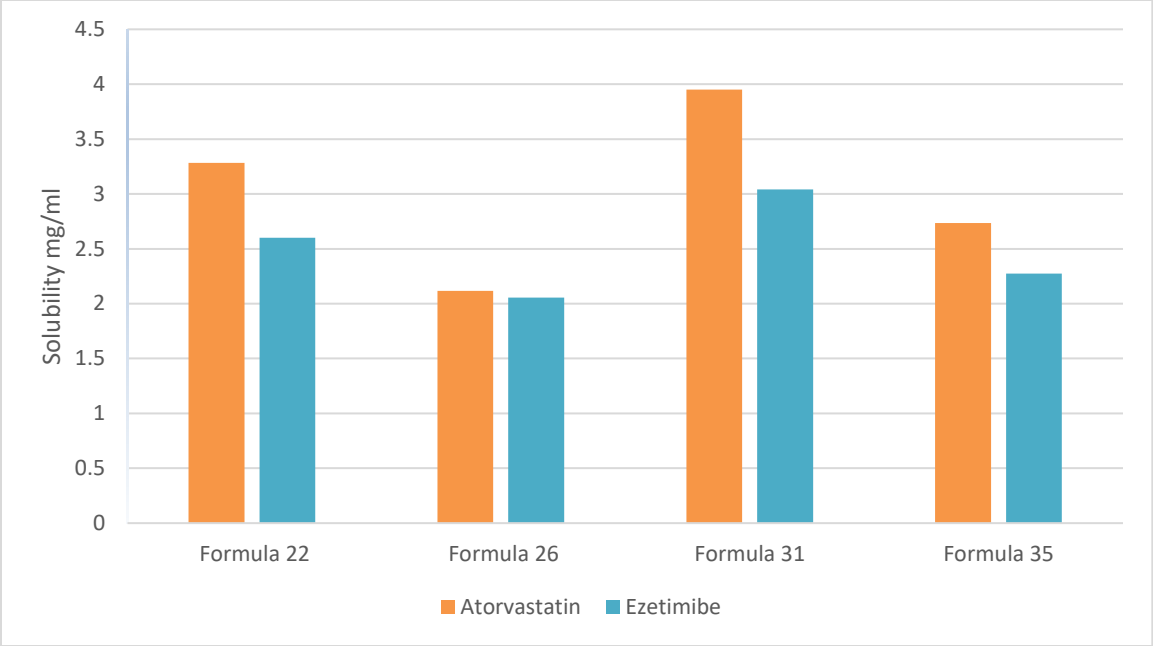


Figure 21: Solubility of ATV and EZE at different micro emulsion

4.8 Stability studies for formulations of micro emulsion without active materials

This step was conducted to determine the stability of selected micro emulsion formulations before adding actives to these formulations. The droplet size of each micro emulsion formulation was measured after freeze-thaw cycle and after one week at room temperature as illustrated in Table 22.

Table 22: Stability studies for formulations of micro emulsion without actives.

Formula number	**Formula Composition**	**Ratio** Oil: surf/co-surf.	**Ratio** Surf: Co-surf.	**Water %**	**Oil %**	**Surf. %**	**co-surf %**	**Droplet size (nm)** at time zero	**Droplet size (nm)** after freeze-thaw cycle	**Droplet size (nm)** after one week at room temp.
ME #22	Triacetin: Tw 80/PG	2:8	2:1	25	14.97	39.9	19.9	51.73±5.29	21.35±1.34	37.93±2.97
ME #26	Triacetin: Tw 80/PG	3:7	3:1	15	25.45	44.66	14.89	16.21±2.03	14.35±0.26	11.51±0.23
ME #31	Triacetin: Tw 80 / PEG 400	2:8	2:1	25	14.96	39.89	19.94	74.15±1.68	62.58±0.22	69.28±1.27
ME #35	Triacetin: Tw 20 / PEG 400	2:8	1:1	25	14.96	29.92	29.92	10.55±2.72	4.72±0.40	4.26±0.40

All the selected formulations of microemulsion were stable and the droplet size of each formulation was within the range of (10 – 100 nm). In addition, all formulations were not separated (monophasic) after the centrifugation stress test (that mentioned in section 3.3.6.5).

Depending on the previous results in Table 22, formulations ME#22 (Triacetin: Tween 80/PG 2:8), ME#26 (Triacetin: Tween 80/PG 3:7), ME#31 (Triacetin: Tween 80 / PEG 400 2:8), were selected to study their stability upon addition of the active materials and to perform in vitro dissolution study on each formula.

4.8 Stability studies of the selected formulations of micro emulsion with active materials

4.8.1 Stability study of the selected micro emulsion formulations with ATV/EZE at room temperature

Stability experiments were carried out on the chosen microemulsion formulations at room temperature for a period of two weeks. The droplet size of each microemulsion formulation was measured using DLS and the percentage of actives in each formulation was determined using HPLC at time zero and every week thereafter.

The results of stability studies at room temperate are illustrated in the following Tables (Table 23, Table 24, and Table 25).

Table 23: Assay % and droplet size of the selected formulations of micro emulsion with actives at Time Zero.

Formula number	Formula Composition	Ratio Oil: surf/co-surf.	Ratio Surf: Co-surf.	Water %	Oil %	Surf. %	co-surf %	ATV Assay %	EZE Assay %	Particle size (nm)	Polydispersity
ME #22	Triacetin: Tw 80/PG	2:8	2:1	25	4.97	39.9	19.9	103.5±1.17	106.5±1.42	68.34±1.24	0.390±0.753
ME #26	Triacetin: Tw 80/PG	3:7	3:1	15	25.45	44.66	14.89	99.4±0.58	102.9±2.07	48.22±0.59	0.068±0.050
ME #31	Triacetin: Tw 80 /PEG 400	2:8	2:1	25	14.96	39.89	19.94	104.6±1.04	104.9±0.91	53.21±0.83	0.372±0.022
ME #35	Triacetin: Tw 20 /PEG 400	2:8	1:1	25	14.96	29.92	29.92	102.5±2.53	107.6±1.22	64.65±0.48	0.2340.035

Table 24: Assay % and droplet size of the selected formulations of micro emulsion with actives after one week at room temperature.

Formula number	Formula Composition	Ratio Oil: surf/co-surf.	Ratio Surf: Co-surf.	Visual appearance	ATV Assay %	EZE Assay %	Particle size (nm)	Polydispersity
ME #22	Triacetin: Tw 80/PG	2:8	2:1	Clear	98.4±3.36	105.31±1.23	72.84±0.37	0.27±0.04
ME #26	Triacetin: Tw 80/PG	3:7	3:1	Clear	99.06±1.05	102.64±1.75	34.52±0.20	0.42±0.24
ME #31	Triacetin: Tw 80 /PEG 400	2:8	2:1	Clear	103.12±1.65	103.22±0.86	79.05±0.31	0.25±0.53
ME #35	Triacetin: Tw 20 /PEG 400	2:8	1:1	Clear	100.52±2.14	103.72±2.80	68.2±0.05	0.16±0.21

Table 25: Assay % and droplet size of the selected formulations of micro emulsion with actives after 2 weeks at room temperature.

Formula number	**Formula Composition**	**Ratio** Oil: surf/co-surf.	**Ratio** Surf: Co-surf.	**Visual appearance**	**ATV Assay %**	**EZE Assay %**	**Particle size (nm)**	**Polydispersity**
ME #22	Triacetin: Tw 80/PG	2:8	2:1	Clear	99.57±0.46	102.25±1.62	68.05±0.26	0.34±0.12
ME #26	Triacetin: Tw80/PG	3:7	3:1	Clear	100.28±1.19	103.87±0.33	31.71±0.41	0.28±0.17
ME #31	Triacetin: Tw 80 /PEG 400	2:8	2:1	Clear	100.71±2.25	104.65±1.63	67.38±0.48	0.45±0.53
ME #35	Triacetin: Tw 20 /PEG 400	2:8	1:1	Clear	100.41±1.27	104.55±0.73	74.52±0.63	0.25±0.44

According to Tables 23,24, and 25, all formulations were stable and has a droplet size within the range (10-100nm).

In addition to that all formulations fell within the assay range (95-105%) as can be seen in Figure 22 below.

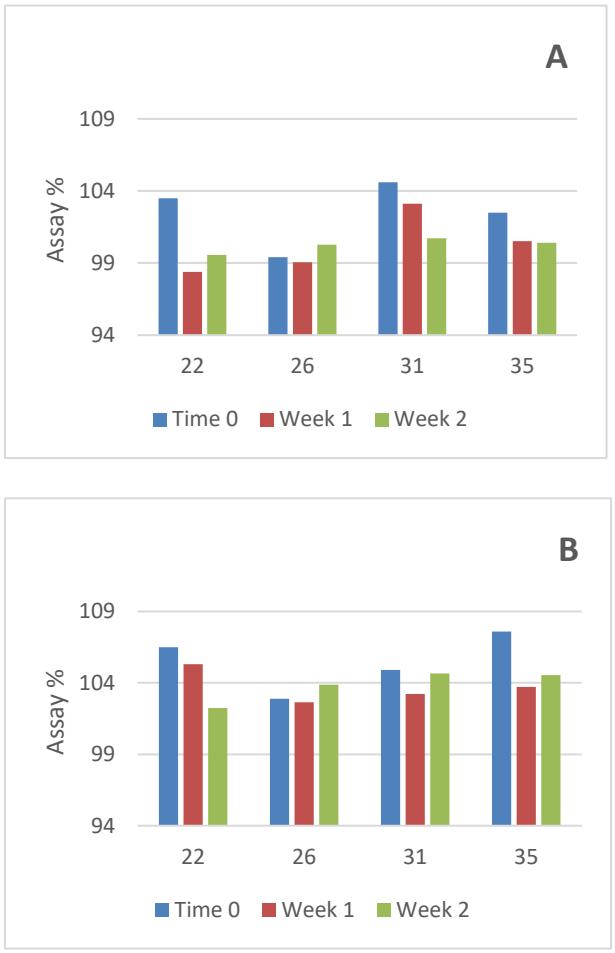


Figure 22: Assay changes at time 0, week 1, and week 2 for (A): ATV, (B): EZE.

4.8.2 Accelerated Stability study for selected formulations of micro emulsion with actives

The accelerated stability of each selected micro emulsion formulation was assessed through freeze-thaw cycle and centrifugation stress tests, as discussed in sections 3.3.6.4 and 3.3.6.5, respectively. The results are presented in Tables 26, and 27.

All formulations remained stable after freeze thaw cycles and centrifugation stress tests, with no degradation.

Table 26: The Assay % and visual appearance of the selected micro emulsion formulations after Freeze-thaw cycle.

Formula number	Formula Composition	Ratio Oil: surf/co-surf.	Ratio Surf: Co-surf.	Visual appearance	actives precipitation	ATV Assay %	EZE Assay%
ME #22	Triacetin: Tw 80/PG	2:8	2:1	Clear monophasic	No	98.24±0.23	101.25±2.61
ME #26	Triacetin: Tw 80/PG	3:7	3:1	Clear monophasic	No	97.19±0.51	99.31±3.65
ME #31	Triacetin: Tw 80 / PEG 400	2:8	2:1	Clear monophasic	No	96.57±0.89	101.34±3.47
ME #35	Triacetin: Tw 20 / PEG 400	2:8	1:1	Clear monophasic	No	98.25±2.26	98.35±2.23

Table 27: The visual appearance of the selected micro emulsion formulations after centrifugation stress test.

Formula number	Formula Composition	Ratio Oil: surf/co-surf.	Ratio Surf: Co-surf.	Visual appearance	Actives precipitation
ME #22	Triacetin: Tw 80/PG	2:8	2:1	Clear monophasic	No
ME #26	Triacetin: Tw 80/PG	3:7	3:1	Clear monophasic	No
ME #31	Triacetin: Tw 80 /PEG 400	2:8	2:1	Clear monophasic	No
ME #35	Triacetin: Tw 20 /PEG 400	2:8	1:1	Clear monophasic	No

4.9 Filling microemulsion in soft gel capsules

When filling microemulsion manually in a soft gel capsule, the process involves carefully measuring the desired density of the microemulsion and using a syringe or pipette to transfer it into the empty capsule shell. The density of the microemulsion formulas have been calculated, dividing mass of the microemulsion by its volume, the calculated density was approximately 1 mg/ml. the formulas have then been filled manually using a pipete in a size 00 of the Empocabs capsules. the capsules contained 40 mg of ATV, 10 mg of EZE in addition to 450 mg of excipients.

After the filling process, the capsules have been subjected to stability testing in addition to in-vitro drug release studies.

4.9.1 Stability study of filled capsules

The capsules have been subjected to stability study at 30°C, 40°C and at the refrigerator for the period of one month.

The results of stability study of samples stored at 30°C, and at the refrigerator showed no remarkable change in drug content which remained above 95% for both of ATV and EZE, indicating that the combination is stable with no drug degradation at both of storage conditions, meanwhile, the capsules stored at 40°C suffered from leakage and couldn't be assayed.

Table 28: The Assay % and visual appearance of the selected micro emulsion formulations after one month stability study

Formula number	**Formula Composition**	**Ratio** Oil: surf/co-surf.	**Ratio** Surf: Co-surf.	**Visual appearance**	**ATV Assay % at 30 C**	**ATV Assay %at Refrigerator**	**EZE Assay % at 30 C**	**EZE Assay %at Refrigerator**
ME #22	Triacetin: Tw 80/PG	2:8	2:1	Clear	95.62±0.16	95.10±0.73	96.32±0.26	98.57±1.05
ME #26	Triacetin: Tw80/PG	3:7	3:1	Clear	95.66±0.32	95.93±1.21	99.54±0.42	98.60±2.13
ME #31	Triacetin: Tw 80 /PEG 400	2:8	2:1	Clear	100.88±0.25	100.69±0.60	97.51±1.04	97.59±0.24
ME #35	Triacetin: Tw 20 / PEG 400	2:8	1:1	Clear	98.46±1.04	97.38±0.51	97.12±1.45	98.38±0.67

4.9.2 In vitro drug release study

4.6.2.1 Dissolution at pH=6.8 for all developed formulas

Figure 23 (A), and (B) represents the dissolution profile comparison between the Brand Atozet and the formulas 22, 26 and 31, showing the dissolution profile at pH=6.8.

The dissolution parameters DE and MDT were calculated. As can be inferred from Table 28, the brand yielded the highest MDT for both of ATV and EZE with values of 9.41 and 8.19 respectively. Whereas, the DE was the lowest with values of 0.68 and 0.72.

Similarity (F2) and difference factors (F1) were also calculated, results are shown in Table 28. All developed formulas have a similar dissolution profile as the brand product, with F2 values greater than 50, and F1 less than 15.

Formula 31 has the lowest mean dissolution time compared to other formulas, which can be credited to the surfactant combination (Tween 80/ PEG 400). Also, formula 31 has the highest percentage of drug release which is particle size dependent. This indicates that smaller drops create larger interfacial area, leading to faster drug release.

Formula 26 has the highest percentage of Triacetin (oily phase) in comparison to other formulas. In addition to having higher percentage of Tween, which explains the higher dissolution efficacy in comparison to other formulas.

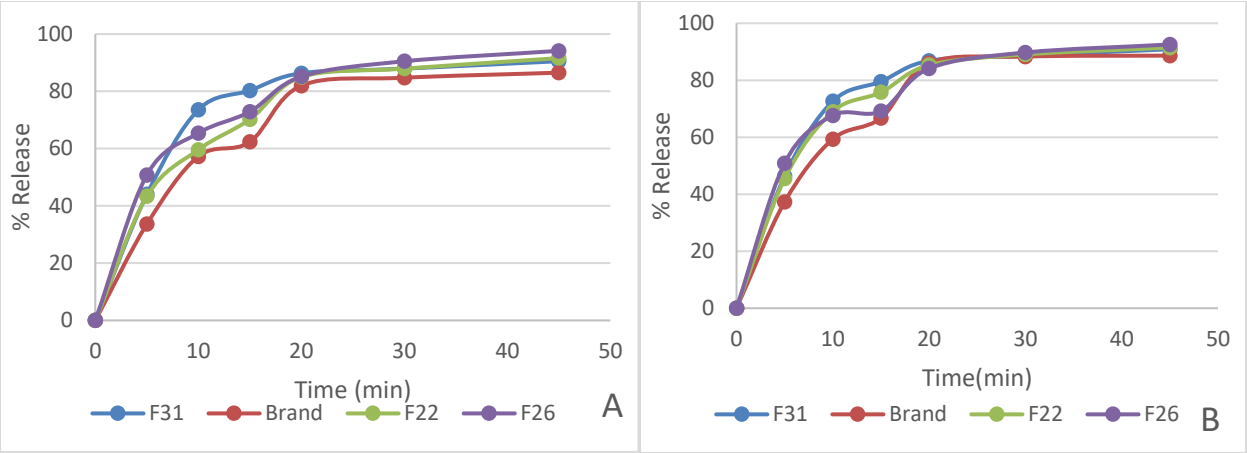


Figure 23: Release% in formulas 22,26 and 31 Vs. The brand at pH=6.8, of (A) Ezetimibe and (B) Atorvastatin.

Table 28 represents the release percentage of with the dissolution parameters: MDT, DE, F1, and F2.

Table 28: Dissolution data of formulas 22, 26, 31 at pH=6.8 for both ATV and EZE

4.6.2.2 Dissolution profile of formula 31

Ezetimibe at pH= 6.8					Atorvastatin at pH= 6.8			
Time	**Brand**	**F31**	**F22**	**F26**	**Brand**	**F31**	**F22**	**F26**
0	0	0	0	0	0	0	0	0
5	33.62±7.7	44 ± 7.8	43.34±8.1	50.78±5.8	37.32±12.8	46.34±12.5	45.47±5.6	50.88±8.5
10	56.03±5.9	63.55 ±6.9	59.6±4.5	65.40±8.0	62.12±6.6	75.961±7.6	69.00±1.1	67.63±2.6
15	63.33±5.4	75.3±5.0	70.22±12.8	72.89±6.5	70.42±10.8	83.5±5.6	75.76±6.8	75.17±3.4
20	80.83±6.4	84.33±4.0	85±3.4	85.18±6.2	86.03±7.9	86.83±7.4	85.3±6.01	84.12±5.1
30	84.75±4.0	87.91±4.7	87.93±0.6	90.49±3.0	88.3±6.5	88.65±6.8	89.06±1.8	89.74±4.5
45	86.51±3.8	90.51±2.9	91.6±3.3	94.14±1.6	88.65±4.6	90.86±5.1	91.45±2.1	92.54±0.7
MDT	9.41	8.27	9.08	8.65	8.19	6.81	7.93	8.09
DE	0.68	0.74	0.73	0.76	0.72	0.77	0.75	0.75
F2		56.00	61.23	50.00		53.00	64.53	59.00
F1		10	8	13		9	6	7

Formula 31 has been selected for further studying due to its superior drug release percentages for both ATV and EZE, as well as its highest solubility.

Table 29 represents the dissolution profile data of formula 31 at three PH's: 1.2, 4.5 and 6.8 vs. the Brand Atozet. Dissolution parameters has been calculated and compared.

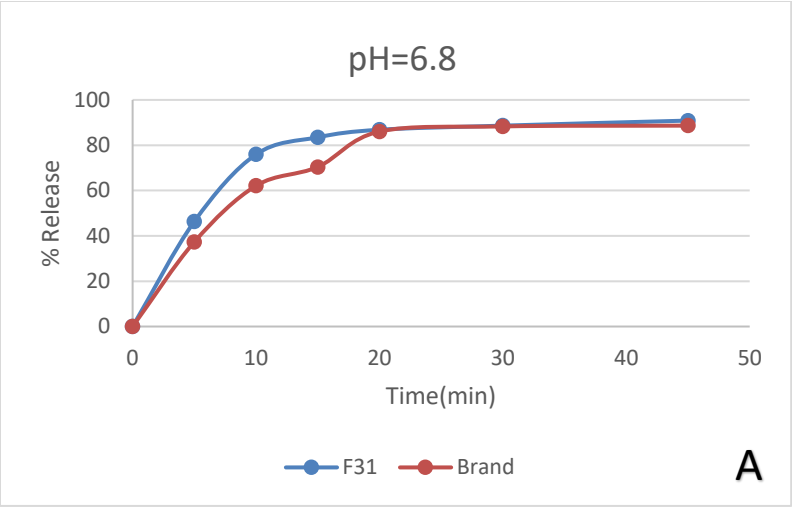
The ionization constant of a drug and the pH of the dissolution medium are crucial factors that influence the solubility of weak acids and bases. The intrinsic solubility refers to the solubility of its free acid or base form, with weak acids exhibiting similar solubility at pH levels slightly lower than their pKa value. A rise in pH leads to increased solubility attributed to the presence of the ionized form [3].

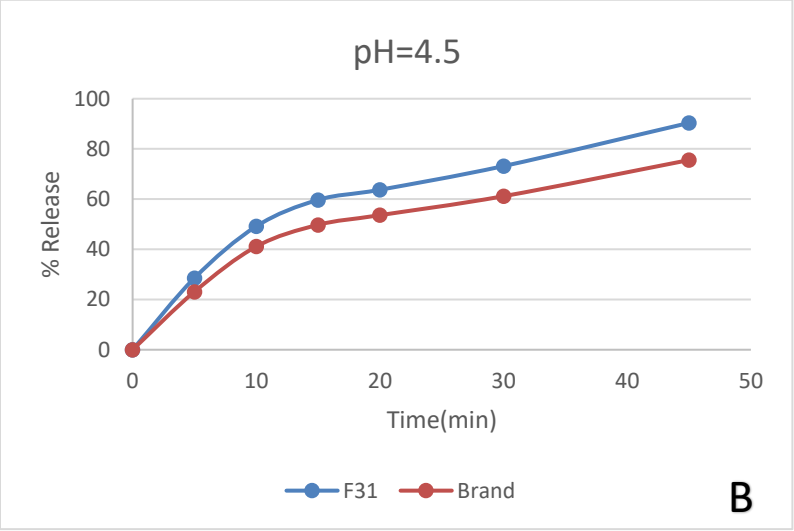
When a drug has low solubility in water at different pH levels, surfactants can be included to dissolution medium in order to enhance the solubilization. Since Ezetimibe is a non-ionizable drug, the pH of the medium does not impact its solubility. Our formula included Tween 80 in its composition which enhanced release rate, still, since Ezetimibe is a weak base and non-ionizable, release at the pH =1.2 was lower than at the two other medias. At pH =6.8 and pH =4.5, both of the actives had higher dissolution rates than at pH=1.2.

When comparing the dissolution parameters of formula 31 and the brand, as can be seen in Table 29, the MDT of formula 31 is lower than that of the brand in all media for both ATV and EZE, meanwhile, the DE is higher also in all media.

Table 29: Dissolution data for formula 31 at PH=1.2, 4.5, and 6.8

	Ezetimibe at pH=6.8		Atorvastatin at pH=6.8		Ezetimibe at pH=4.5		Atorvastatin at pH=4.5		Ezetimibe at pH=1.2		Atorvastatin at pH=1.2	
Time	**F31**	**Brand**	**F31**	**Brand**	**F31**	**Brand**	**F31**	**Brand**	**F31**	**Brand**	**F31**	**Brand**
0	0	0	0	0	0	0	0	0	0	0	0	0
5	44 ± 7.8	33.62±7.7	46.34±12.5	37.32±12.8	35.28±8.5	28.63±8.4	28.55±4.7	23.1±4.7	45.57±11.7	29.77±10.8	9.03±10.8	6.77±14.7
10	73.55 ±6.9	56.03±5.9	75.961±7.6	62.12±6.6	50.18±2.9	41.95±2.9	49.16±2.5	41.11±2.6	45.67±3.5	30.39±0.5	13.47±1.7	8.77±5.6
15	80.3±5.0	63.33±5.4	83.5±5.6	70.42±10.8	62.93±4.0	52.6±3.9	59.7±1.7	49.75±1.7	47.07±4.6	30.90±0.5	20.42±3.1	12.67±4.7
20	86.33±4.0	80.83±6.4	86.83±7.4	86.03±7.9	82.77±2.8	69.2±2.8	63.9±0.1	53.6±0.1	47.78±4.4	31.40±0.5	21.94±6.6	13.85±2.3
30	87.91±4.7	84.75±4.0	88.65±6.8	88.3±6.5	84.36±2.3	70.21±2.3	73.16±1.5	61.16±1.5	49.00±6.6	31.92±0.5	22.31±7.0	14.97±0.7
45	90.51±2.9	86.51±3.8	90.86±5.1	88.65±4.6	88.81±1.3	74.25±1.4	90.36±2.2	75.56±2.2	49.41±1.9	33.67±4.2	23.67±1.3	15.81±1.7
MDT	7.27	9.40	6.81	8.19	10.28	10.39	14.48	14.52	3.85	5.13	9.70	10.16
DE	0.75	0.68	0.77	0.72	0.68	0.57	0.61	0.51	0.45	0.29	0.18	0.12
F2	48		53		47		49		40		58	
F1	14		9		20		20		51		52	





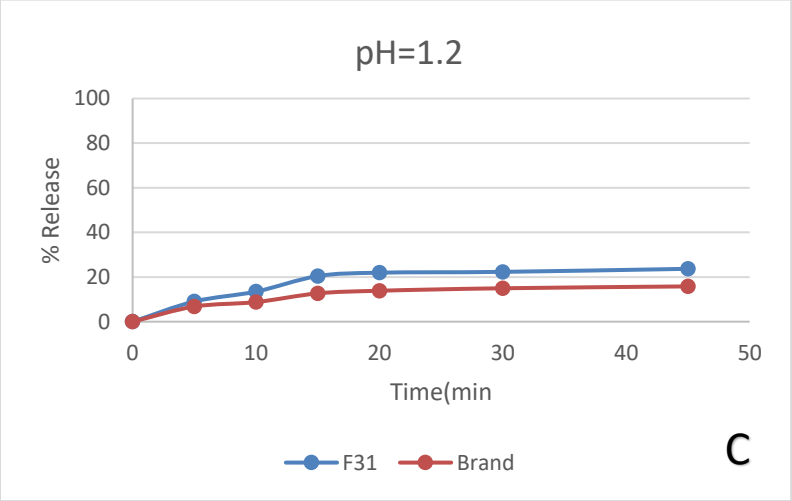
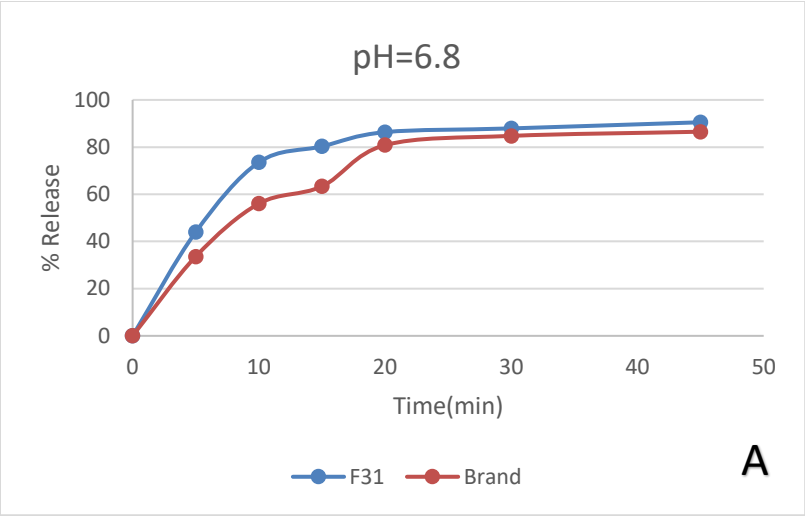
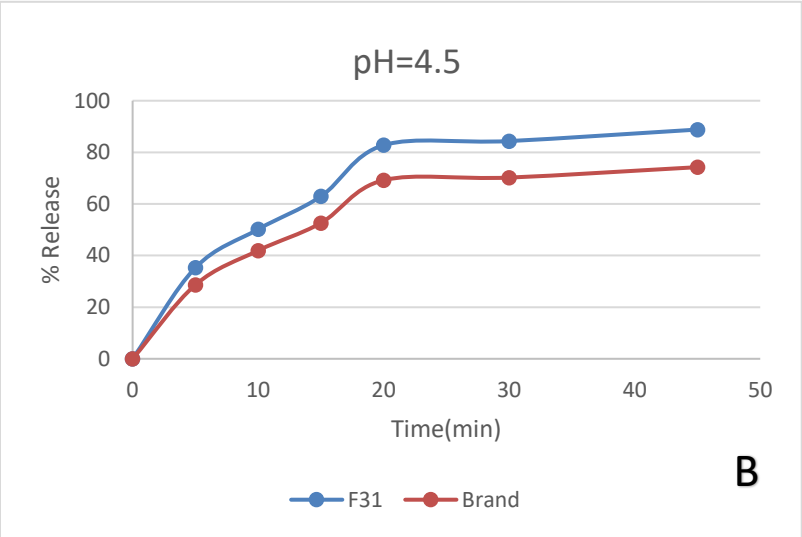


Figure 24: Atorvastatin of formula 31 Vs. Brand: (A): at pH =6.8, (B): at pH =4.5, (C): at pH=1.2





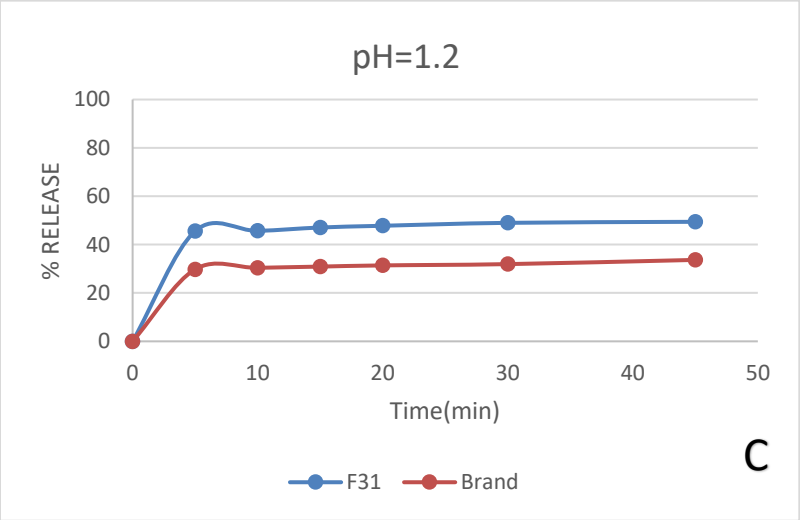


Figure 25: Ezetimibe of formula 31 Vs. Brand: (A): at pH =6.8, (B): at pH =4.5, (C): at pH=1.2

Since our formula contains Tween 80, while the brand doesn't-which aid the dissolution of the actives- our formula had better dissolution efficacy than the brand.

Also, the increased drug release rate from our formulation in comparison to the Brand, may be due to the relationship between quantitative drug release and droplet size in developed microemulsion. Which suggests that microemulsion with smaller drops have a higher interfacial area, resulting in faster drug release.

As a result, formula 31 with the composition of: Triacetin= 14.94%, Tween 80= 39.89%, PEG 400= 19.94, water=25% and physical characteristics as follows:

Viscosity (Cp)	Refractive index	Droplet size(nm)	Polydispersity index	Assay	Dissolution
310±9.58	1.372	74.15±1.68	0.34±0.02	Within range	Within range

In addition to a solubility of Atorvastatin of: 3.95 mg/ml and solubility of Ezetimibe of: 3.04 mg/ml, was chosen as the optimum formula.

Figures 26 and 27 below, illustrates individually formula 31 ATV and EZE in comparison to Brand ATV and EZE in all media.

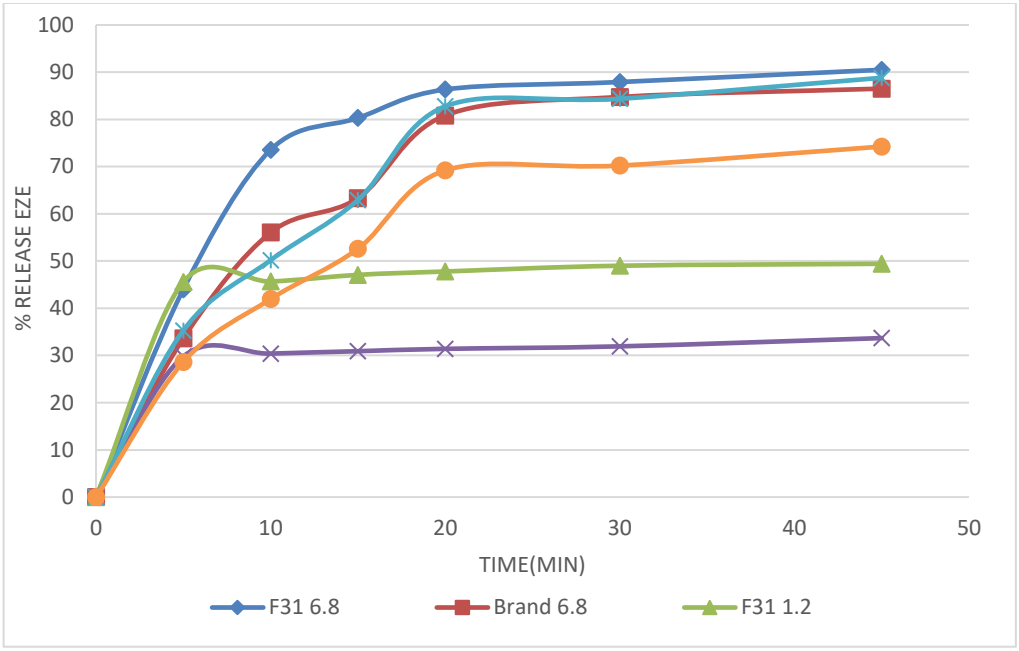


Figure 26: Formula 31 EZE vs Brand EZE dissolution profile at pH=6.8

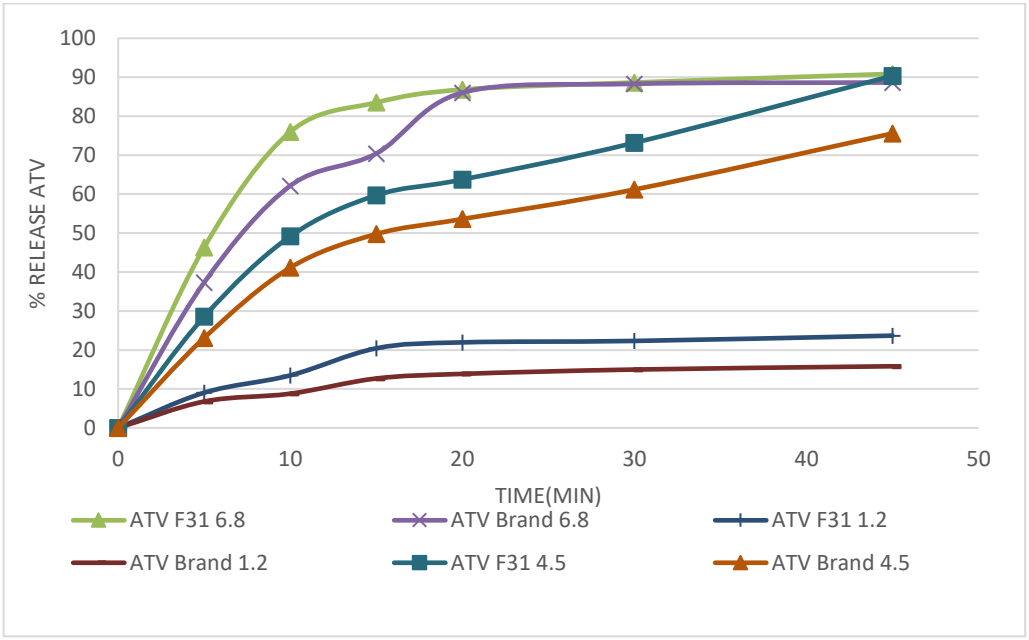


Figure 27: Formula 31 ATV vs Brand ATV dissolution profile at pH=6.8

Figure 28 illustrates the difference of the ATV and Ezetimibe in all three media in comparison to the brand.

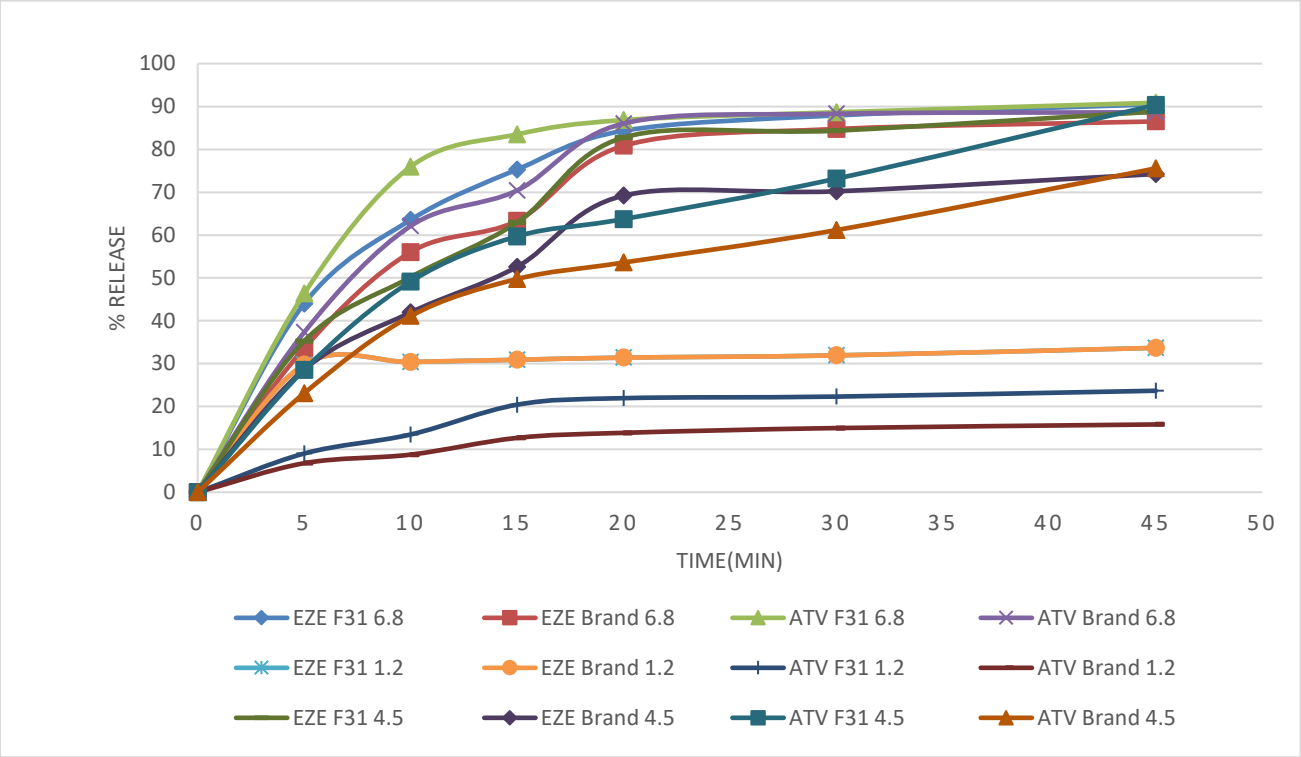


Figure 28: Dissolution Figure of formula 31 at PH=6.8

Conclusion

Conclusion

The study resulted in the successful development of an optimized formulation for ATV/EZE loaded SMEDDS encapsulated in a soft gel capsule. The enhanced formula included 10 mg of ezetimibe, 40 mg of Atorvastatin, 14.96% Triacetin (oil), 39.89% Tween 80, and 19.94% PEG 400. This composition produced a stable microemulsion when exposed to an aqueous medium, despite any changes in pH levels.

In-vitro release trials indicated that the improved formula exhibited swifter release compared to pure drugs and commercially available products, confirming the effectiveness of soft gel capsule of SMEDDS in enhancing the solubility and dissolution speed of poorly water-soluble drug combinations (ATV/EZE).

The improved systemic absorption of medications from optimized soft gel capsule of SMEDDs can be linked to the combination of surfactants used (Tween 80/ PEG 400), as these bio enhancer surfactants have been shown to increase drug bioavailability by enhancing transcellular absorption and reducing intestinal efflux.

Future work

Moving forward, it is essential to continue the assessment of the long-term stability of the microemulsion formulations to determine their shelf life and potential degradation over extended periods of 3 and 6 months. Furthermore, conducting additional animal studies to evaluate the in-vivo performance and therapeutic efficacy of the ATV/EZT soft gel capsules. The antihyperlipidemic effect of ATV/EZT soft gel capsule is to be investigated using poloxamer-induced hyperlipidemic rat model. Poloxamer 407 is a non-ionic surfactant that is known to cause a rapid onset of hyperlipidemia after single intraperitoneal dose will provide comprehensive insights into their clinical potential. Exploring the potential for scale-up and commercial production of these microemulsion-based formulations will be crucial for their translation into practical pharmaceutical applications.

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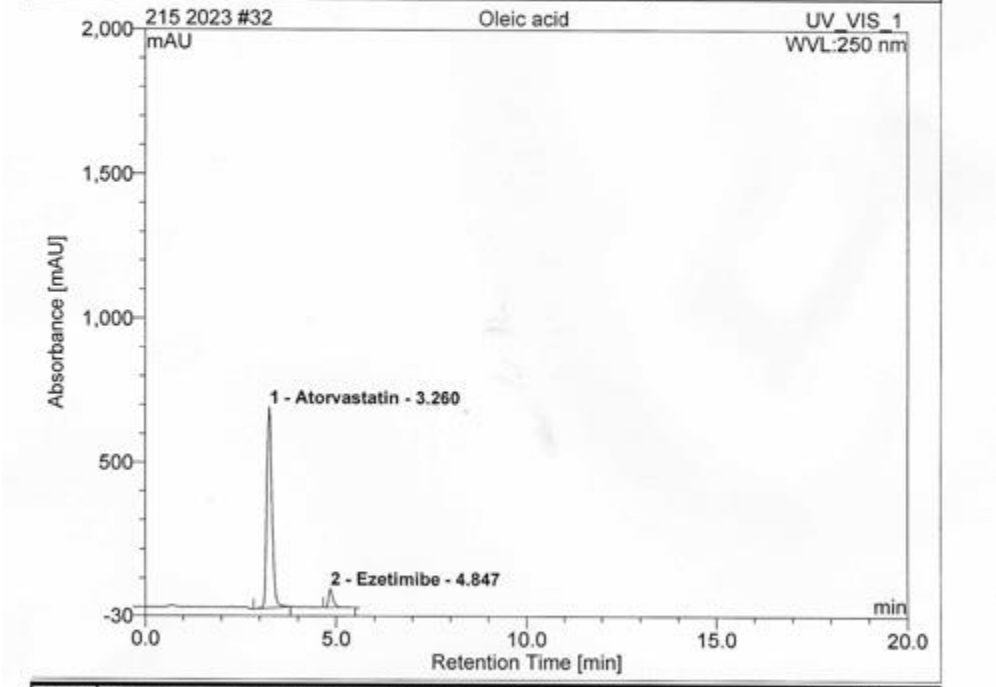
Appendices

Appendix A: Solubility chromatograms in various oils

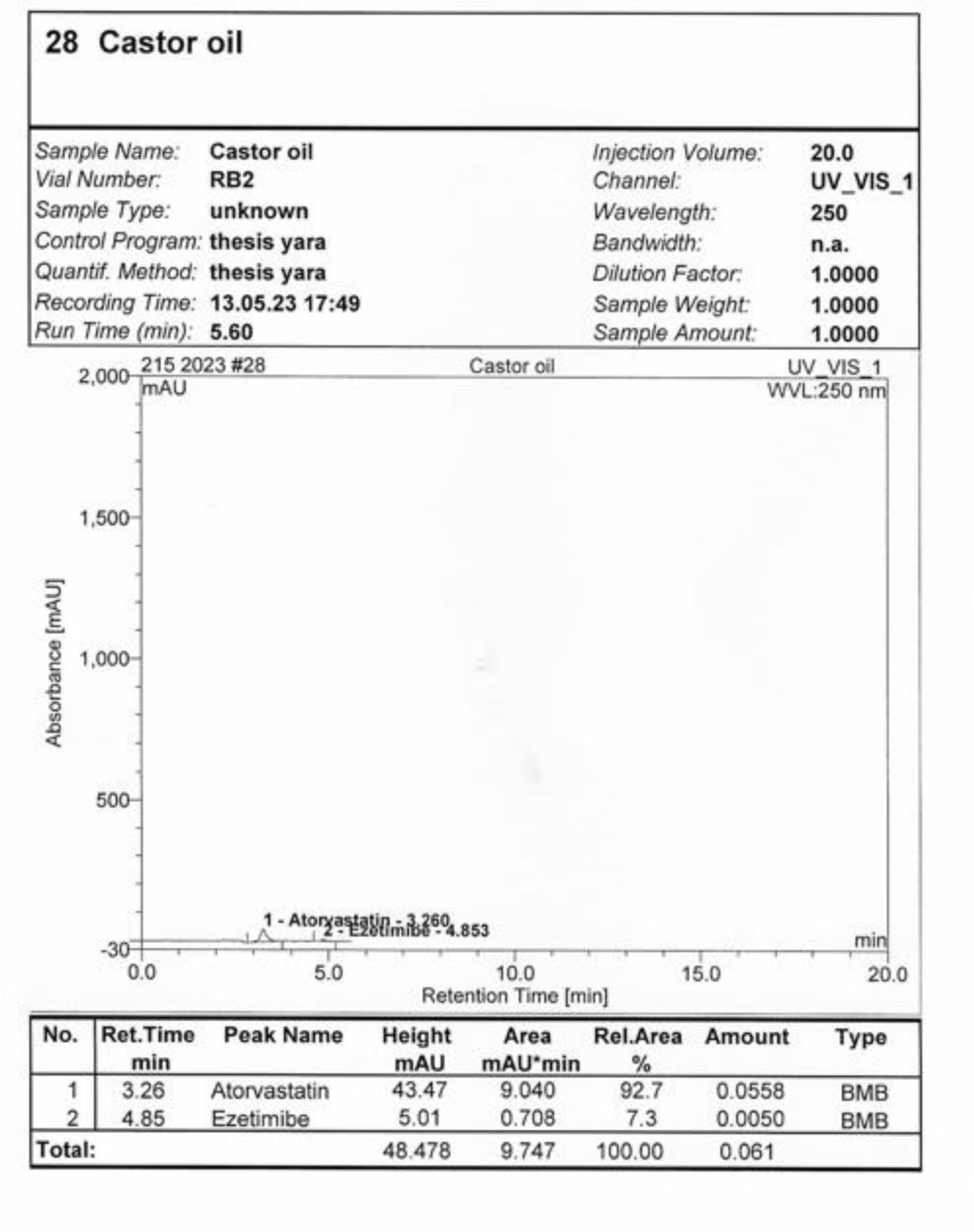
32 Oleic acid

Sample Name:	**Oleic acid**	*Injection Volume:*	**20.0**
Vial Number:	**RB3**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**250**
Control Program:	**thesis yara**	*Bandwidth:*	**n.a.**
Quantif. Method:	**thesis yara**	*Dilution Factor:*	**1.0000**
Recording Time:	**13.05.23 18:15**	*Sample Weight:*	**1.0000**
Run Time (min):	**5.60**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.26	Atorvastatin	695.83	109.114	92.9	0.6730	BMB
2	4.85	Ezetimibe	63.95	8.290	7.1	0.0581	BMB
Total:			759.774	117.403	100.00	0.731	

Figure A1: solubility chromatogram in oleic acid

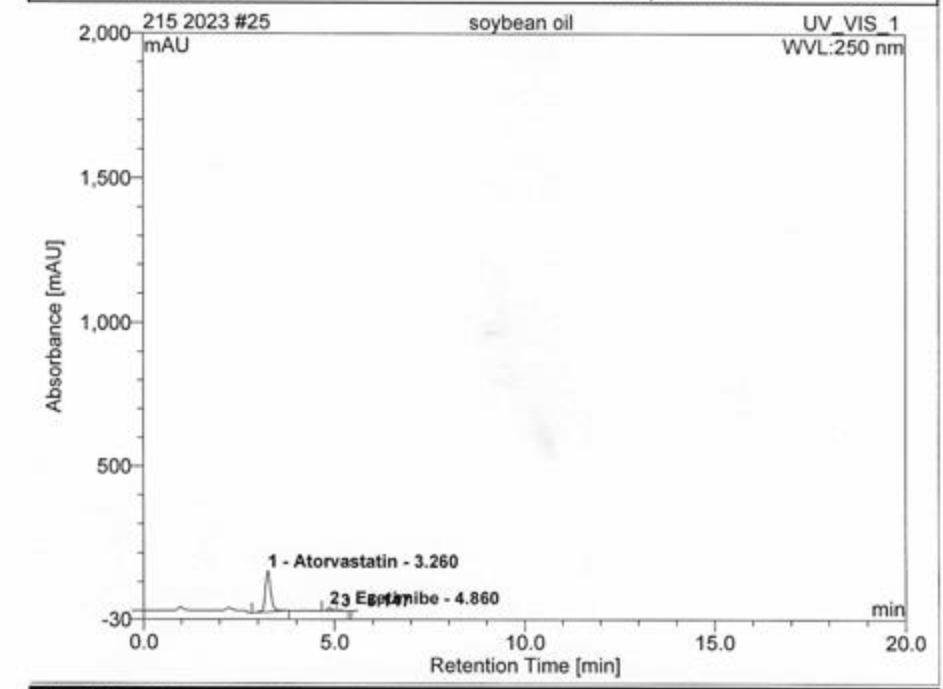


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.26	Atorvastatin	43.47	9.040	92.7	0.0558	BMB
2	4.85	Ezetimibe	5.01	0.708	7.3	0.0050	BMB
Total:			48.478	9.747	100.00	0.061	

Figure A2: solubility chromatogram in castor oil

25 soybean oil

Sample Name:	**soybean oil**	*Injection Volume:*	**20.0**
Vial Number:	**RA7**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**250**
Control Program:	**thesis yara**	*Bandwidth:*	**n.a.**
Quantif. Method:	**thesis yara**	*Dilution Factor:*	**1.0000**
Recording Time:	**13.05.23 17:30**	*Sample Weight:*	**1.0000**
Run Time (min):	**5.60**	*Sample Amount:*	**1.0000**

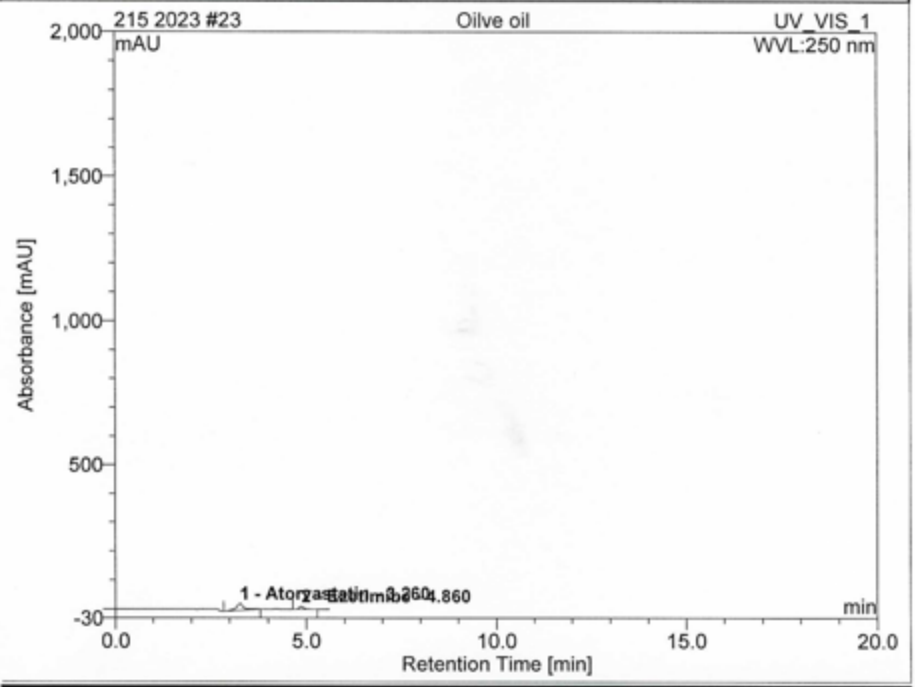


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.26	Atorvastatin	143.16	23.730	93.2	0.1464	BMB
2	4.86	Ezetimibe	10.84	1.512	5.9	0.0106	BMB
3	5.15	n.a.	1.83	0.225	0.9	n.a.	Rd
Total:			155.819	25.467	100.00	0.157	

Figure A3: solubility chromatogram in soybean oil

23 Oilve oil

Sample Name:	**Oilve oil**	*Injection Volume:*	**20.0**
Vial Number:	**RA6**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**250**
Control Program:	**thesis yara**	*Bandwidth:*	**n.a.**
Quantif. Method:	**thesis yara**	*Dilution Factor:*	**1.0000**
Recording Time:	**13.05.23 17:17**	*Sample Weight:*	**1.0000**
Run Time (min):	**5.60**	*Sample Amount:*	**1.0000**

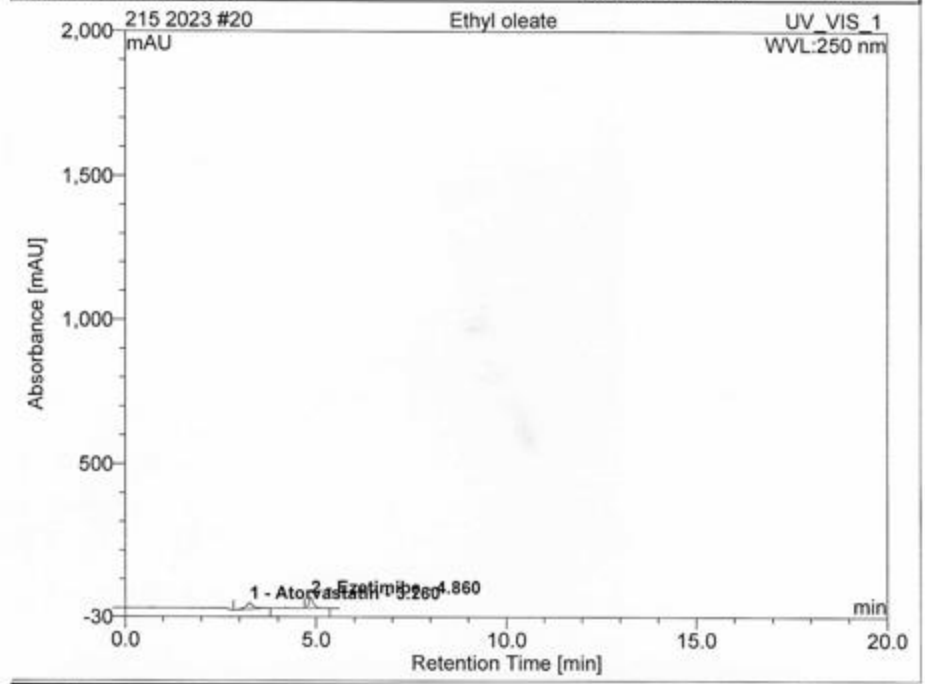


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.26	Atorvastatin	23.51	5.500	82.0	0.0339	BMB
2	4.86	Ezetimibe	9.24	1.205	18.0	0.0085	BMB
Total:			32.742	6.705	100.00	0.042	

Figure A4: solubility chromatogram in olive oil

20 Ethyl oleate

Sample Name:	**Ethyl oleate**	*Injection Volume:*	**20.0**
Vial Number:	**RA5**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**250**
Control Program:	**thesis yara**	*Bandwidth:*	**n.a.**
Quantif. Method:	**thesis yara**	*Dilution Factor:*	**1.0000**
Recording Time:	**13.05.23 16:58**	*Sample Weight:*	**1.0000**
Run Time (min):	**5.60**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.26	Atorvastatin	22.19	5.312	51.8	0.0328	BMB
2	4.86	Ezetimibe	38.21	4.934	48.2	0.0346	BMB
Total:			60.399	10.245	100.00	0.067	

Figure A5: solubility chromatogram in ethyl oleate

Appendix B: microemulsion formulations properties

Table B1: Micro emulsion formulations properties for Oleic acid: Tween 80/ PG with ratios of 1:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B2: Micro emulsion formulations properties for Oleic acid: Tween 80/ PEG 400 with ratios of 1:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

Table B3: Micro emulsion formulations properties for Oleic acid: Koll RH 40/ PG with ratios of 1:1 of oil: Sur mix.														
Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B4: Micro emulsion formulations properties for Oleic acid: Koll RH 40/ PEG 400 with ratios of 1:1 of oil: Sur mix.														
Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B5: Micro emulsion formulations properties for Oleic acid: Tween 20/ PG with ratios of 1:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B6: Micro emulsion formulations properties for Oleic acid: Tween 20/ PEG 400 with ratios of 1:1 of oil: Sur mix.														
Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf. **2:8**	☑	×	×	☑	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B7: Micro emulsion formulations properties for Oleic acid: Tween 80/ PG with ratios of 1:2 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf. **2:8**	×	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B8: Micro emulsion formulations properties for Oleic acid: Tween 80/ PG with ratios of 2:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	×	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B9: Micro emulsion formulations properties for Oleic acid: Tween 80/ PG with ratios of 1:3 of oil: Sur mix.														
Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B10: Micro emulsion formulations properties for Oleic acid: Tween 80/ PG with ratios of 3:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B11: Micro emulsion formulations properties for Oleic acid: Tween 80/ PEG 400 with ratios of 1:2 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	×	×	×	×	×	☑	☑	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B12: Micro emulsion formulations properties for Oleic acid: Tween 80/ PEG 400 with ratios of 2:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	×	×	×	☑	☑	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	×	☑	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B13: Micro emulsion formulations properties for Oleic acid: Tween 80/ PEG 400 with ratios of 1:3 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B14: Micro emulsion formulations properties for Oleic acid: Tween 80/ PEG 400 with ratios of 3:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	□	□	□	□	□	□	□	□	□	□	□	□	□	□

The symbol ☑means that the formulation was clear and monophasicwhile the

Table B15: Micro emulsion formulations properties for Oleic acid: KOLL RH 40/ PEG 400 with ratios of 2:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

symbol × means that the formulation was turbid or witnessed phase separation.

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B16: Micro emulsion formulations properties for Oleic acid: KOLL RH 40/ PEG 400 with ratios of 1:2 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	×	×	☑	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	☑	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B17: Microemulsion formulations properties for Oleic acid: KOLL RH 40/ PEG 400 with ratios of 1:3 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	×	×	×	☑	☑	☑	☑	☑	☑	☑	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	×	☑	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B18: Micro emulsion formulations properties for Oleic acid: KOLL RH 40/ PEG 400 with ratios of 3:1of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B19: Micro emulsion formulations properties for Oleic acid: Tween 20/ PG with ratios of 1:2 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

Table B20: Micro emulsion formulations properties for Oleic acid: Tween 20/ PG with ratios of 2:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	☑	☑	☑	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

symbol × means that the formulation was turbid or witnessed phase separation.

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B21: Micro emulsion formulations properties for Oleic acid: Tween 20/ PG with ratios of 1:3 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B22: Micro emulsion formulations properties for Oleic acid: Tween 20/ PG with ratios of 3:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	☑	☑	☑	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The following are the Tables related to the other oily phase which is Triacetin

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B23: Micro emulsion formulations properties for Triacetin: Tween 80/ PG with ratios of 1:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B24: Micro emulsion formulations properties for Triacetin: Tween 80/ PG with ratios of 1:2 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B25: Micro emulsion formulations properties for Triacetin: Tween 80/ PG with ratios of 2:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	☑	☑	×	×	×	×	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B26: Micro emulsion formulations properties for Triacetin: Tween 80/ PG with ratios of 1:3 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	☑	☑	×	×	×	×	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B27: Micro emulsion formulations properties for Triacetin: Tween 80/ PG with ratios of 3:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	☑	☑	×	×	×	×	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B28: Micro emulsion formulations properties for Triacetin: Tween 80/ PEG 400 with ratios of 1:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	×	×	☑×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
9:1	×	×	×	×	×	×	×	×	×	×	×	×	×	×

Table B29: Micro emulsion formulations properties for Triacetin: Tween 80/ PEG 400 with ratios of 1:2 of oil: Sur mix.														
Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	×	×	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	×	×	×	×	×	×	×	×	×	×	×	×	×	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B30: Micro emulsion formulations properties for Triacetin: Tween 80/ PEG 400 with ratios of 2:1 of oil: Sur mix.														
Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B31: Micro emulsion formulations properties for Triacetin: Tween 80/ PEG 400 with ratios of 1:3 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	×	×	×	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	×	×	×	×	×	×	×	×	×	×	×	×	×	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B32: Micro emulsion formulations properties for Triacetin: Tween 80/ PEG 400 with ratios of 3:1 of oil: Sur mix.														
Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	×	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B33: Micro emulsion formulations properties for Triacetin: KOLL RH 40/ PG with ratios of 1:1 of oil: Sur mix.														
Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B34: Micro emulsion formulations properties for Triacetin: KOLL RH 40/ PEG 400 with ratios of 1:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B35: Micro emulsion formulations properties for Triacetin: Tween 20/ PG with ratios of 1:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
Oil: surf/co-surf **1:9**	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
oil %	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
Oil: surf/co-surf**. 2:8**	☑	☑	☑	☑	☑	☑	×	×	×	×	×	×	☑	☑
oil %	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
Oil: surf/co-surf**. 3:7**	☑	☑	☑	☑	☑	×	×	×	×	×	×	×	×	×
oil %	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
Oil: surf/co-surf**. 4:6**	☑	☑	☑	×	×	×	×	×	×	×	×	×	×	×
oil %	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
Oil: surf/co-surf**. 5:5**	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
oil %	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
Oil: surf/co-surf**. 6:4**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
Oil: surf/co-surf**. 7:3**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
Oil: surf/co-surf**. 8:2**	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
oil %	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05
Oil: surf/co-surf**. 9:1**	×	×	×	×	×	×	×	×	×	×	×	×	×	×

The symbol ☑means that the formulation was clear and monophasicwhile the

symbol × means that the formulation was turbid or witnessed phase separation.

Table B36: Micro emulsion formulations properties for Triacetin: Tween 20/ PEG 400 with ratios of 1:1 of oil: Sur mix.

Water %	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**	**55%**	**60%**	**65%**	**70%**
water(µl)	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
oil %	**132**	**146**	**163**	**184**	**208**	**238**	**275**	**321**	**379**	**455**	**556**	**694**	**893**	**1190**
Oil: surf/co-surf **1:9**	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
oil %	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
Oil: surf/co-surf**. 2:8**	19.00	18.00	17.00	16.00	15.00	14.00	13.00	12.00	11.00	10.00	9.00	8.00	7.00	6.00
oil %	☑	☑	☑	☑	☑	×	×	×	×	×	×	☑	☑	☑
Oil: surf/co-surf**. 3:7**	28.50	27.00	25.50	24.00	22.50	21.00	19.50	18.00	16.50	15.00	13.50	12.00	10.50	9.00
oil %	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
Oil: surf/co-surf**. 4:6**	37.99	36.00	34.00	32.00	30.00	28.00	26.02	24.00	22.00	20.00	18.00	16.00	14.00	12.00
oil %	☑	☑	×	×	×	×	×	×	×	×	×	×	×	×
Oil: surf/co-surf**. 5:5**	47.49	45.00	42.50	40.00	37.51	35.01	32.51	30.01	27.52	25.02	22.52	20.03	17.53	15.03
oil %	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
Oil: surf/co-surf**. 6:4**	56.99	54.00	51.00	48.00	45.01	42.01	39.01	36.01	33.02	30.02	27.02	24.03	21.03	18.03
oil %	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
Oil: surf/co-surf**. 7:3**	66.49	62.99	59.50	56.00	52.51	49.01	45.51	42.02	38.52	35.03	31.53	28.03	24.54	21.04
oil %	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Oil: surf/co-surf**. 8:2**	75.99	71.99	68.00	64.00	60.01	56.01	52.02	48.02	44.03	40.03	36.04	32.04	28.05	24.05
oil %	☑	×	×	×	×	×	×	×	×	×	×	×	×	×
Oil: surf/co-surf**. 9:1**	85.49	80.99	76.50	72.00	67.51	63.01	58.52	54.02	49.53	45.03	40.54	36.04	31.55	27.05

Appendix C: Pseudo ternary phase diagrams

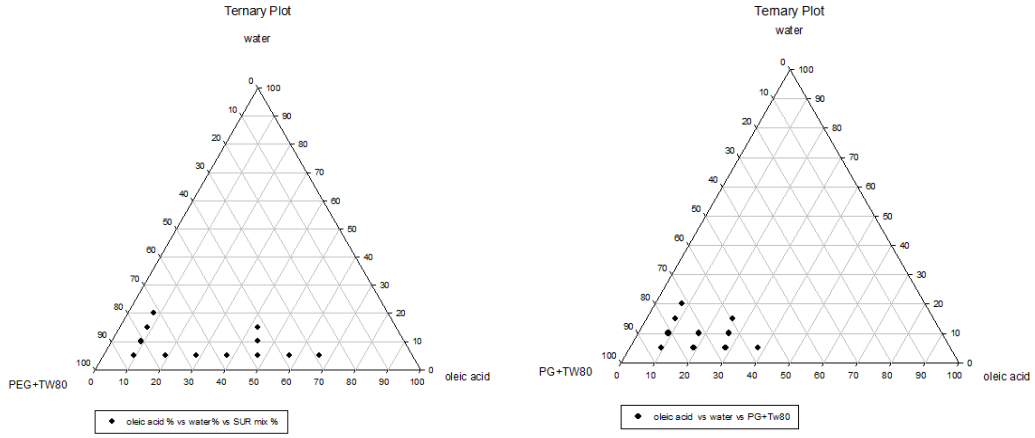


Figure C1: Pseudo ternary phase diagram of Oleic acid+ water+ PEG/TW80 (1:1), and Pseudo ternary phase diagram of Oleic acid+ water+ PG/TW80 (1:1) respectively

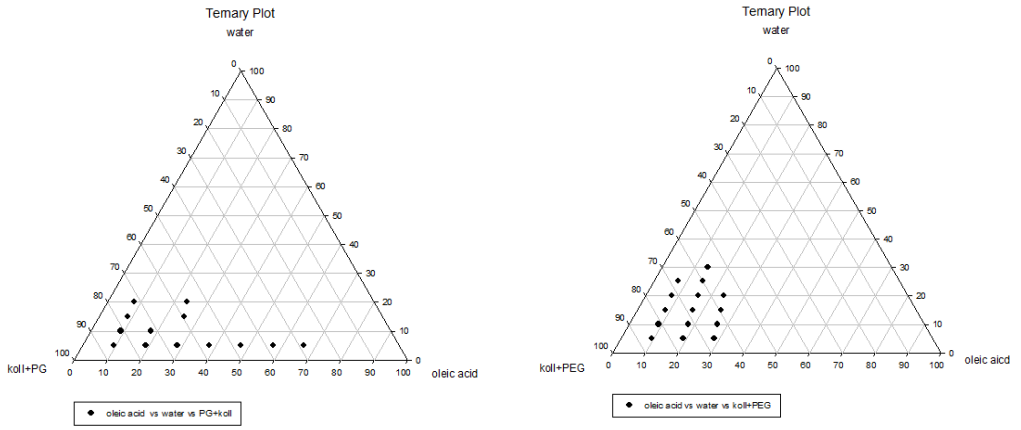


Figure C2: Pseudo ternary phase diagram of Oleic acid+ water+ PG/Koll RH (1:1), Pseudo ternary phase diagram of Oleic acid+ water+ PEG/Koll RH (1:1) respectively

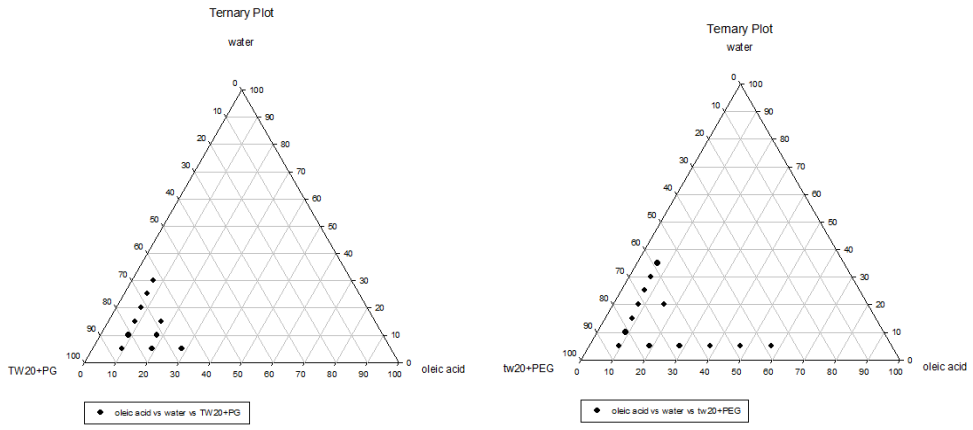


Figure C3: Pseudo ternary phase diagram of Oleic acid+ water+ PG/TW20 (1:1), Pseudo ternary phase diagram of Oleic acid+ water+ PEG/TW20 (1:1) respectively

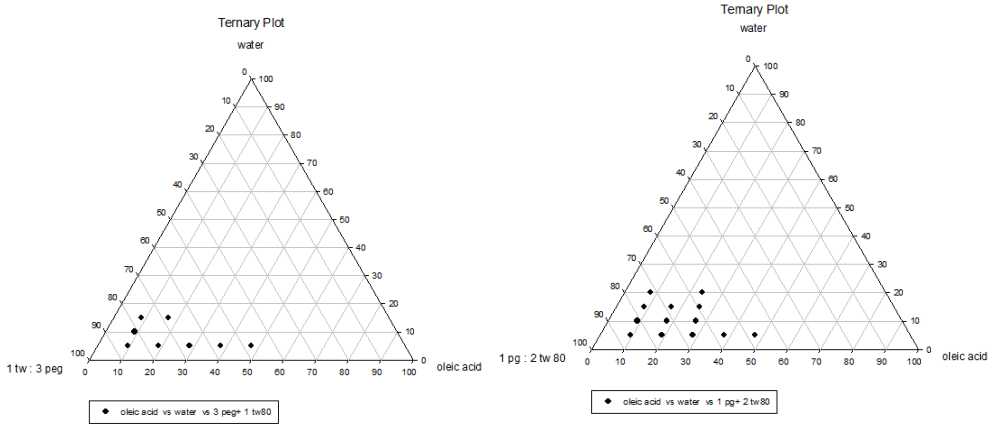


Figure C4: Pseudo ternary phase diagram of Oleic acid+ water+ PEG 400/TW 80 (3:1), Pseudo ternary phase diagram of Oleic acid+ water+ PG 400/TW 80 (1:2) respectively

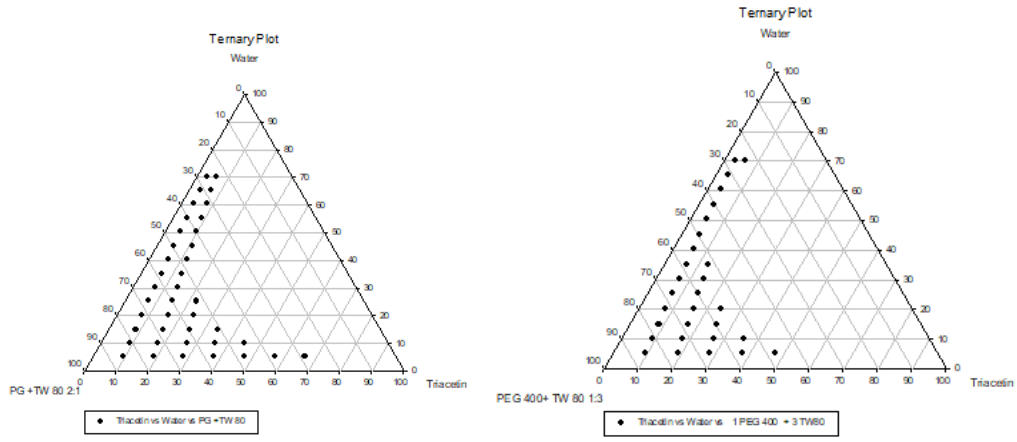


Figure C5: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (2:1), Pseudo ternary phase diagram of Triacetin+ water+ PEG400 /TW 80 (1:3)

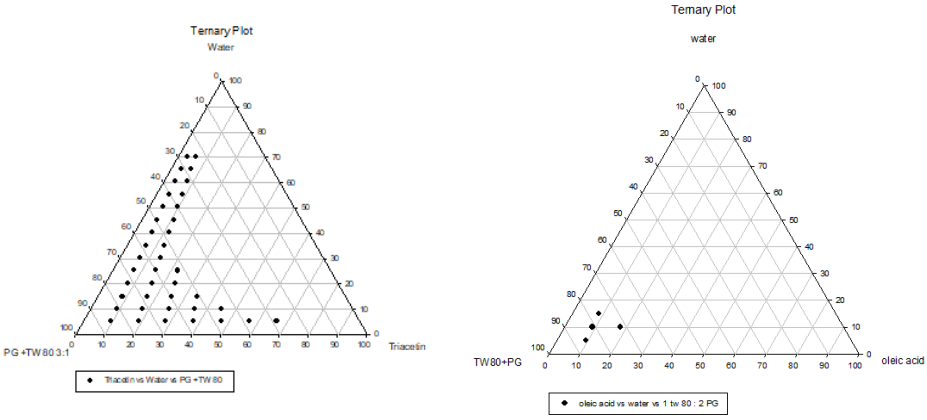


Figure C6: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of oleic acid+ water+ PG /TW 80 (2:1), respectively

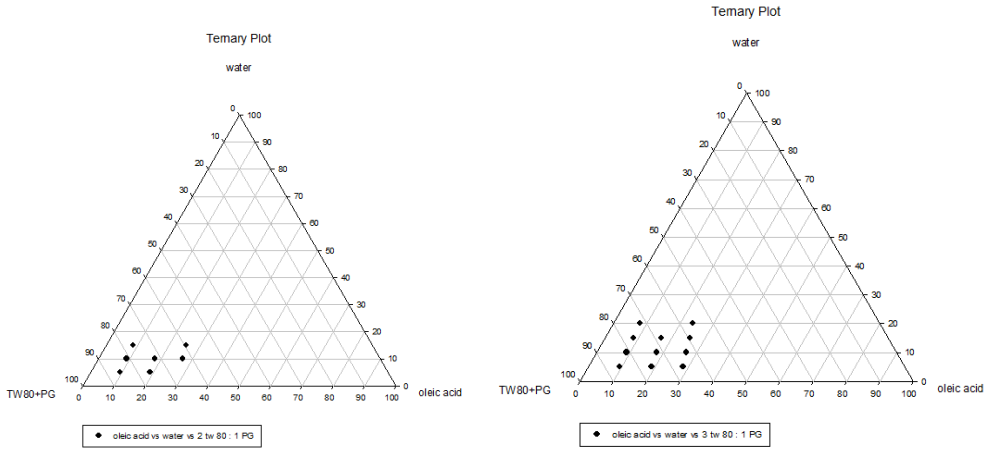


Figure C7: Pseudo ternary phase diagram of oleic acid+ water+ PG /TW 80 (1:2), Pseudo ternary phase diagram of oleic acid+ water+ PG /TW 80 (1:3) respectively

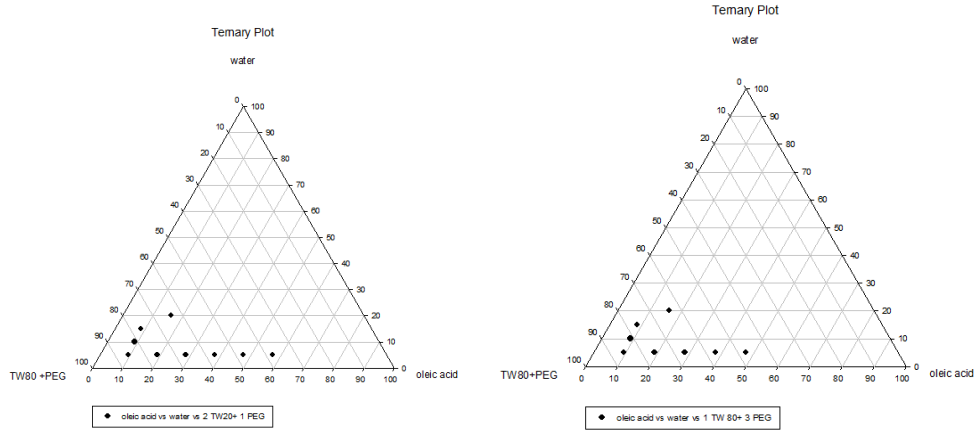


Figure C8: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1) respectively

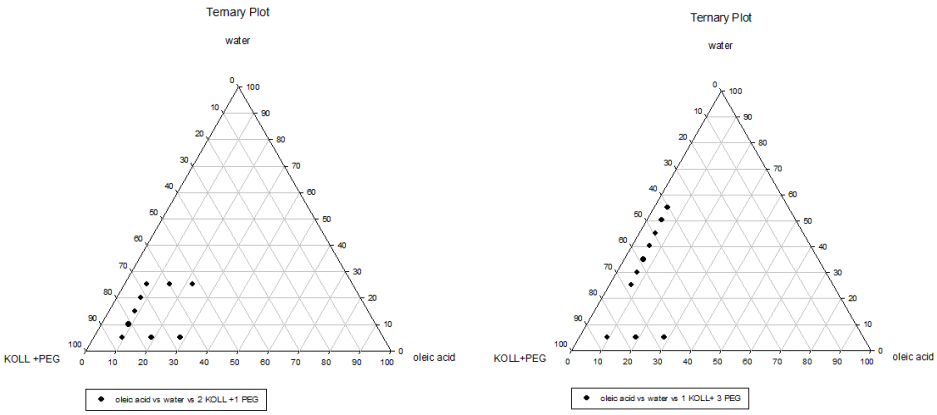


Figure C9: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1)

respectively

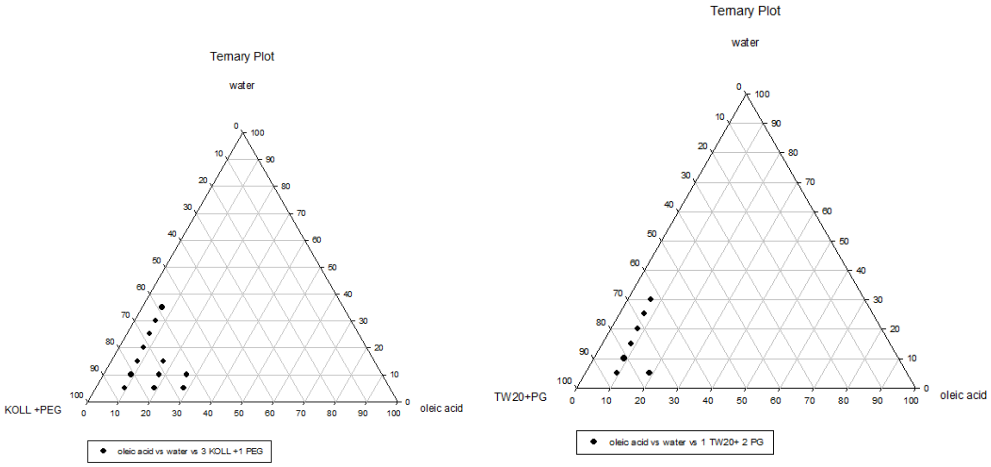


Figure C10: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1) respectively

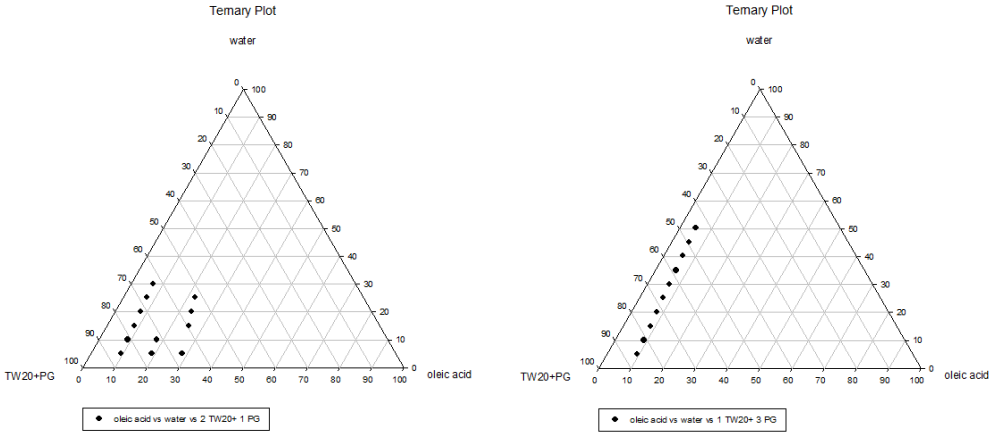


Figure C11: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1) respectively

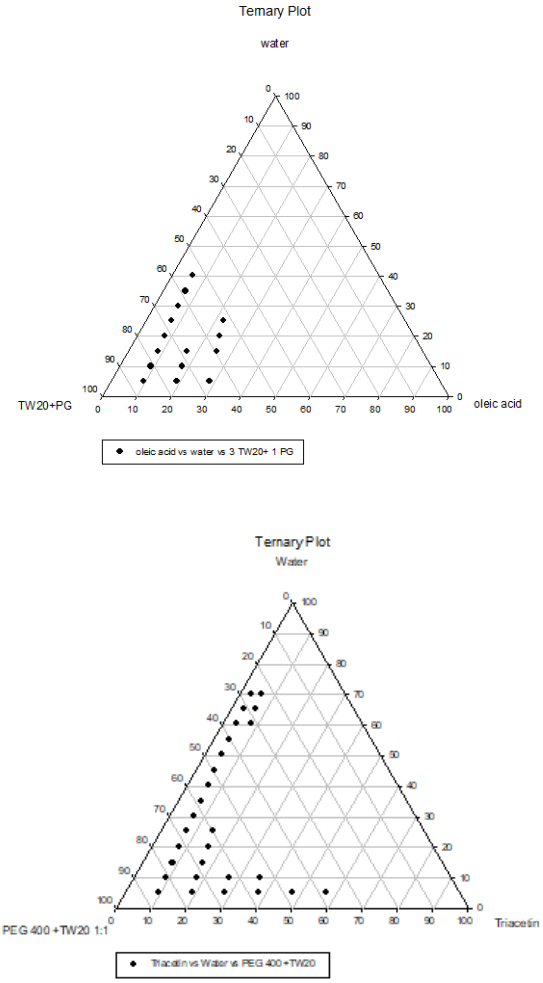


Figure C12: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1) respectively

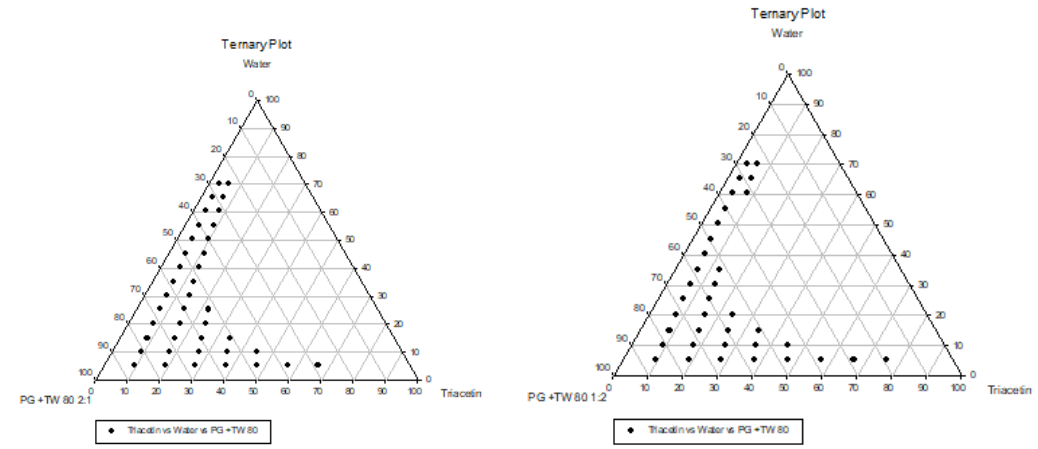


Figure C13: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80

(3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1) respectively

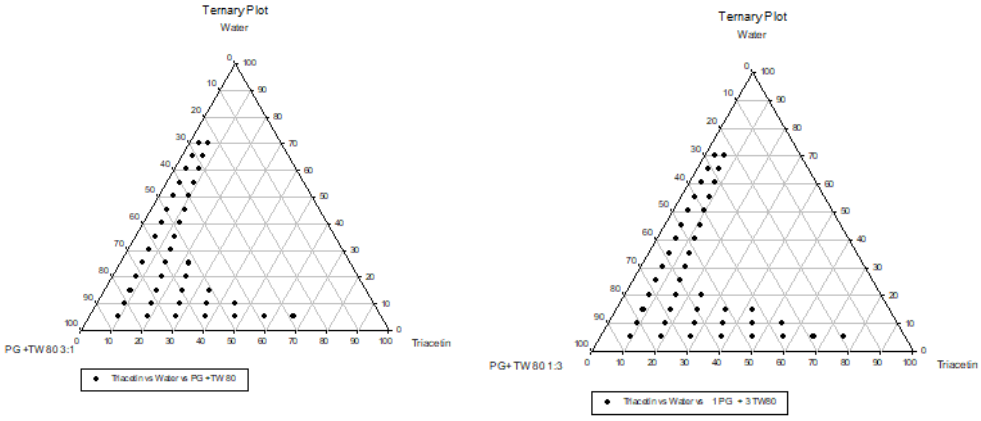


Figure C14: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1) respectively

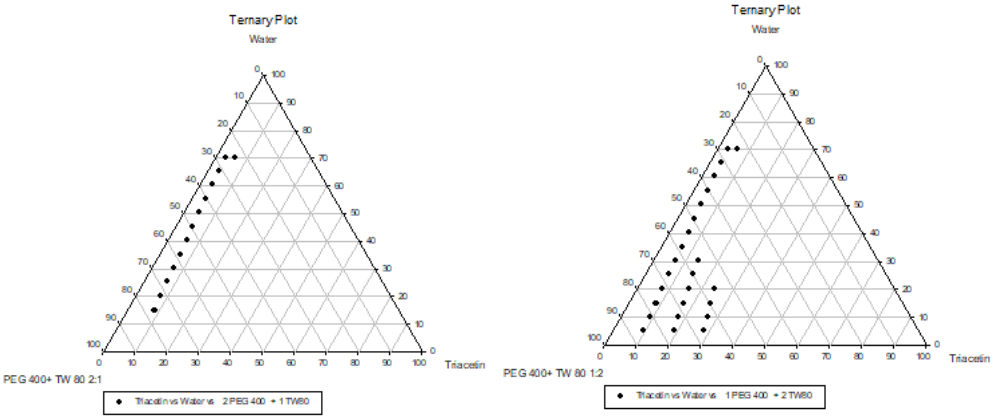


Figure C15: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1) respectively

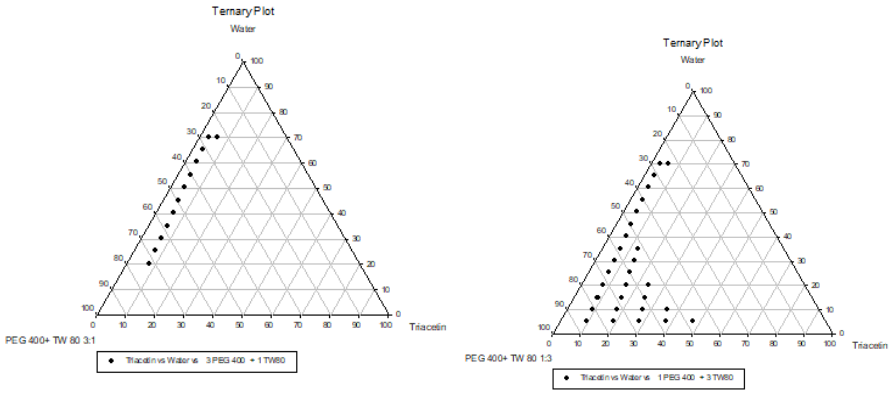


Figure C16: Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1), Pseudo ternary phase diagram of Triacetin+ water+ PG /TW 80 (3:1) respectively