Comparison and Evaluation of Aceclofenac Nanoemulsion Formulation Using Homogenization and Ultrasonication Techniques

By
Kazi Mustari Jahan Saiky
ID : 15146097

A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

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Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

06/07/2020

Kazi Mustari Jahan Saiky
15146097

Approval

The thesis/project titled "Comparison and Evaluation of Aceclofenac Nanoemulsion Formulation Using Homogenization and Ultrasonication Techniques" submitted by Kazi Mustari Jahan Saiky (15146097) of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on February 27th, 2020.

Examining Committee:

Supervisor:

(Member)

Marzia Alam
Lecturer, Department of Pharmacy
Brac University

Program Coordinator:

(Member)

Dr. Hasina Yasmin
Professor, Department of Pharmacy
Brac University

Departmental Head:

(Chair)

Dr. Eva Rahman Kabir
Professor, Department of Pharmacy
Brac University

Ethics Statement

This project involves no human or animal trial.

Abstract

The goal of this study is to evaluate aceclofenac nanoemulsion formulations with varying surfactant-co-surfactant ratio and method of preparation. Aceclofenac is a BCS class II NSAID, with greater anti-inflammatory action than traditional NSAIDS. Ten formulations were prepared in this study, using aceclofenac as the active pharmaceutical ingredient. All 10 formulations contained same amount of aceclofenac, which was 1.5%. Percentage of all the other reagents were kept constant as well except for Tween-80 and ethanol (surfactant and co-surfactant). F1, F2, F3, F4 and F5 were formulated through homogenization method, whereas F1′, F2′, F3′, F4′ and F5′ were formulated through ultrasonication method. All 10 formulations remained stable after 14 days. To further evaluate the formulations, studies like dye test, dilution test, conductivity test, fluorescence test was done. The techniques successfully resulted in 10 stable emulsions, but more studies, e.g. droplet size analysis, zeta potential test, percent transmittance, in-vitro analysis can further confirm if the formulations are nanoemulsions.

Keywords : Aceclofenac; nanoemulsion; ultrasonication; homogenization; anti-inflammatory; Smix

Dedication

To my parents and teachers.

Acknowledgement

First of all, all praise to the almighty Allah.

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List of Acronyms

API	Active pharmaceutical ingredient
O/W	Oil in water
W/O	Water in oil
PEG-400	Polyethylene glycol
PDI	Polydispersity index

Chapter 1

Introduction

Emulsification technology has become more efficient and diverse, moving far beyond early mechanical methods. A recent addition to this are nanoemulsions. These have been a topic of interest because of their efficient delivery of lipid soluble drugs, which always has been a challenge. Terms like mini-emulsion, sub-micron emulsion(SME) and ultra-fine emulsion are some synonyms that are used often (Hakemi-Vala et al., 2017). Nanoemulsions are biphasic systems that are isotropic, kinetically stable and contain two immiscible liquid phases (Jaiswal et al., 2015). One phase is finely dispersed in the continuous phase with a droplet size range of 20-200 nm (Jaiswal et al., 2015). The small size of droplets subdue the unification of droplets and also prevents the precipitation in nanoemulsions (Pagar & Darekar, 2019). This colloidal system requires an emulsifying agent because of its thermodynamically unstable nature. This lack of stability is a result of high interfacial tension present among the two immiscible phases, which most often happen to be oil and water. This tension is stabilized by a surfactant film that coats the droplets. The emulsifying agent may also be referred to as internal phase(Jaiswal et al., 2015).

Nanoemulsions operate as carriers in drug delivery. These carriers are solid spheres with a lipophilic and amorphous surface containing a negative charge (Chircov & Grumezescu, 2019). Nanoemulsions improve drugs solubility and therapeutic activity along with a significant reduction in adverse or toxic effects (Sharma et al., 2013).

As a result of their small size, nanoemulsions are transparent. Nanoemulsion formulations can be of two types : i) O/W nanoemulsion which has oil dispersed in the continuous aqueous phase, ii) W/O nanoemulsion having water dispersed in the oil phase and iii) bi-continuous nanoemulsions where both water and oil are the continuous phases (Jaiswal et al., 2015).

The primary difference between microemulsions and nanoemulsions is not their particle size rather their stability. Microemulsions are thermodynamically stable whereas nanoemulsions are kinetically stable, which is why use of a surfactant is very crucial in nanoemulsion preparation (Fajardo et al., 2016). Surfactant molecules consist of two parts: the lyophilic part causes interaction with the continuous phase. On the other hand, the lyophobic part links with the dispersed phase, hence reducing the surface tension and stabilizing the system.

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) with distinct anti-inflammatory and analgesic properties. Aceclofenac reportedly has a greater anti-inflammatory action than traditional NSAIDS in double blind studies. It is used to treat patients with osteoarthritis and rheumatoid arthritis (Patel & Patel, 2017). This is a BCS class II drug, having high permeability and low solubility (Dasgupta et al., 2013). Oral dosage form of aceclofenac has its severe downsides including gastrointestinal ulcer. Aceclofenac also has its advantages such as significant efficacy, site specific drug administration. So it is safe to say that topical dosage form of aceclofenac might be a fitter solution for long term treatment (Dasgupta et al., 2013).

1.1 Advantages of Nanoemulsions

The greatest advantage of nanoemulsions is that they are safer for use. They provide higher surface area and better absorption because of their small droplet size (Jaiswal et al., 2015). The dosage form comes with a wide range of variation including foams, creams, liquids and sprays. The solubility of lipophilic drugs are enhanced by nanoemulsions (Jaiswal et al., 2015). They also help to mask the unpleasant taste of oral delivery drugs (Sharma et al., 2013). Nanoemulsions are not susceptible to attack by water and air. They are non-toxic and non-irritant.

The smaller droplet size of nanoemulsions gives them the edge over other emulsions. Nanoemulsions are not likely to exhibit problems such as creaming and sedimentation like other emulsions (Pagar & Darekar, 2019). This happens because the gravitational force does not have much impact on the droplets in nanoemulsions as opposed to other kinds of emulsions. Nanoemulsions have higher dispersibility in comparison to microemulsions as small droplet size prevents flocculation (Pagar & Darekar, 2019). Nanoemulsions provides large area of droplets that causes the active ingredients to penetrate via the skin rapidly (Pagar & Darekar, 2019). Nanoemulsion is often found to easily pass through rough skin. This criteria reduces the need of a special penetration enhancer that causes incompatibility. They require less surfactant than microemulsions. Nanoemulsions are transperant and have a fluidy texture. This increases patient compliance and is safe for administration due to the lack of any thickening agent and colloidal particles (Pagar & Darekar, 2019). Nanoemulsions can have various routes of administration including oral, topical, parenteral, nasal and ocular (Simonazzi et al., 2018).

1.2 Limitations of Nanoemulsions

Despite offering great advantages for patients as a delivery system, the small size of droplets is often the reason why the use of nanoemulsion formulations is controlled.

Manufacture of nanoemulsions require droplet size reduction which is a high-cost process because it involves special instruments and techniques e.g. homogenization, micro-fluidization and ultrasonication (Pagar & Darekar, 2019).

Maintaining the stability of nanoemulsions has always been a challenge. This poses a major issue when long-term storage is concerned. Ostwald ripening works as a key factor to indicate the stability of nanoemulsions or lack thereof. Ostwald ripening occurs by dissolving matter in regions with a small curvature radius and by re-precipitation in regions with a large curvature radius. Ostwald ripening results in the dissolution of smaller solid grains, the distribution of the solute through the liquid and the re-precipitation of the solid into large grains. The result is growth in net grain, which in this case is flocculation and or creaming (Pagar & Darekar, 2019).

Convenient surfactant and cosurfactant necessary for nanoemulsion production are not in abundance, which can be indicated as a limitation.

1.3 Components of Nanoemulsion

The primary components for nanoemulsions are oil, emulsifying agents and aqueous phase (Savale et al., 2018). Oils of any kind, such as castor oil, coconut oil, corn oil, evening primrose oil, linseed oil, mineral oil, olive oil and peanut oil can be used. A mixture of oil and water may create a crude temporary emulsion, which is divided in two distinct phases by the coalescence of the scattered globules. Emulgents can provide stability this kind of systems. Emulgents in general are categorized as surfactants like spans and tweens, hydrophilic colloids such as acacia and finely divided solids, e.g., bentonite and veegum(Jaiswal et al., 2015). The emulgent should be non-toxic in addition to its emulsifying properties and should be compliant with the product's taste, odor and chemical stability(Jaiswal et al., 2015). A list of desired criteria for an emulgent is as follows :

- ability to reduce the surface tension to below 10 dynes/cm
- rapid adsorbance around a distributed phase globule to create a complete and coherent film to avoid coalescence
- aid a sufficient zeta potential and viscosity in the system so as to ensure outstanding stability
- operative at a reasonably low concentration

Cosurfactants like ethanol, propylene glycol, and polyethylene glycol-400 (PEG-400), triethanolamine, glycerine are also used to improve the activity of the emulgent (Savale et al., 2018).

1.4 Method of Preparation For Nanoemulsion

The core objectives of the nanoemulsion preparation include the achievement of droplet size range of 100-600 nm. Additionally, another goal is to provide the stability condition (Sharma, Bansal, Visht, Sharma, & Kulkarni, 2010). This requires energy. Nanoemulsions can be prepared using two methods : 1. high-energy mathods - and 2. low-energy methods.

1.4.1 High Energy Methods

High energy methods require mechanical devices that can yield large quantities of enrgy. Devices like microfluidizers, homogenizers and ultrasonic devices can provide that forming small oil droplets (Jasmina et al., 2017). In consequence, with escalating input of energy, reduction in the size of droplet occurs.

1.4.1.1 High Pressure Homogenization

One of the high energy methods used for preparation of O/W nanoemulsions is high pressure homogenization. This method is mostly used to produce finely dispersed emulsions on a continuous basis (Dumay et al., 2013). Pressure is used in this technique to coerce a liquid to pass through a valve which is designed specifically for homogenization, creating particles suspended with a uniform size distribution (Chircov & Grumezescu, 2019). Homogenizers are widely used in food, cosmetics and pharmaceutical industries (Patrignani and Lanciotti, 2016).

1.4.1.2 Microfluidization

Microfluidization is a proprietary mixing technique. A microfluidizer system is used in this method: the macroemulsion passes an interaction chamber due to high pressure resulting in nanoemulsions containing particles that are of sub-micron range. Uniformly dispersed droplets can be obtained repeating the procedure. (Jaiswal et al., 2015).

1.4.1.3 Ultrasonication

Ultrasonication is widely used since it is one of the most cost-effective methods. It is easy to use as well. (Sugumar et al., 2016). This technique includes high-intensity ultrasonic waves generating shear forces, high pressure and temperature by cavitation, leading to an alteration in the properties of the liquid. The process causes both oil and aqueous phase to disrupte and blend into small dispersed oil droplets (Rodriguez-Burneo et al., 2017).

1.4.2 Low Energy Methods

Low energy methods are based on the system's properties and its complex interfacial hydrodynamic mechanisms (Jasmina et al., 2017). The techniques are categorized as either thermal or isothermal. Surfactant properties can alter depending on temperature leading to formation of emulsion, which is utilized in the thermal methods. On the other hand, isothermal methods work with alteration in constant temperature, forming emulsions.

1.4.2.1 Spontaneous Nano emulsification

This process works in room temperature and doesn't require any extraordinary machineries. First, a homogenous organic solution is prepared which contains a mixture of oil and lipophilic surfactant with water miscible solvent and hydrophilic surfactant. Then under constant magnetic

stirring, the organic phase is gradually added to the aqueous phase, resulting in an o/w emulsion. Finally elimination of the aqueous phase via evaporation under reduced pressure is done. (Solans et al. 2005; Tadros et al. 2004)

1.4.2.2 Phase Inversion Methods

These techniques use the chemical energy that is discharged due to the phase transition in emulsification process (Thakur, Walia, & Kumar, 2013). Appropriate transitions in phase can be created either by causing change to the composition at constant temperature or vice versa. A sudden drop in the temperature of the mixture of oil, water, and surfactant below the phase inversion temperature under continuous mixing results in the formulation of emulsion (Komaiko and McClements, 2014).

1.5 Evaluation of Nanoemulsion

Evaluation of nanoemulsion is necessary for better understanding of the system and further improvement. Some other significances include :

- To understand the physical stability of nanoemulsions
- To determine the absorption and colloidal stability of nanoemulsions
- To explore the formation and charecteristics of the nanoemulsion
- Selection of excipients
- To predict manufacture and production process. (Suyal, n.d.)

1.6 Evaluation Paramaters of Nanoemulsion

1.6.1 Droplet Size Analysis

Droplet size distribution of the nanoemulsion can be determined by using a photon correlation spectroscopy. The fluctuations in light scattering caused by Brownian motion of the particles is analyzed by the device, using a Zetasizer (Savale et al., 2018). Observation of light scattering is done at 25°C at a 90° angle.

1.6.2 Stability test

Observation of nanoemulsions can be performed under naked eye. Flocculation, creaming, coalescence and sedimentation are indication of instability.

1.6.3 pH Analysis

pH analysis is a key measurement because of the interaction of nanoemulsions with biological tissues and can be performed with a pH meter or a pH pen (Dasgupta and Ranjan, 2018).

1.6.4 Dye Test

Water soluble dyes are added to the nanoemulsions. If the nanoemulsion is w/o type, the dye will appear only in the dispersed phase under microscopic observation. On the contrary, the entire nanoemulsion will appear evenly dyed under microscope, if the formulation is o/w type. (Jaiswal et al., 2015)

1.6.5 Viscosity determination

Viscosity can be calculated at various shear levels and temperatures using the Brookfield-type rotary viscometer (Jaiswal et al., 2015).

1.6.6 Refractive Index

Refractive index is measured using a refractometer.

1.6.7 Dilution Test

The nanoemulsions are diluted with various solvents and then observed.

1.6.8 Filter Paper Test

Nanoemulsion is poured into a filter paper and observed. An o/w formulation will stretch out rapidly whereas a w/o formulation will move slowly (Sharma and Jain 1985).

1.6.9 Zeta potential

Nanoemulsion is diluted for calculating zeta potential, and its value is derived from oil droplets' electrophoretic mobility. It is measured by an instrument called Zeta PALS. (Yilmaz & Borchert, 2005).

1.6.10 Fluorescence Test

Most oils, when exposed to UV light, display fluorescence. When introduced to a fluorescence light under a microscope, the field of w/o nanoemulsion completely fluoresces. Contrarily, in case of an o/w nanoemulsion, the fluorescence gives a spotted appearance. (Jaiswal et al., 2015).

1.6.11 Polydispersity Index

PDI specifies the droplet uniformity of nanoemulsions and is estimated using a spectrophotometer. High polydispersity index indicates low uniformity of droplet size(Jaiswal et al., 2015).

1.6.12 Percent transmittance

It is measured using a UV-visible spectrophotometer at a certain wavelength (Jaiswal et al., 2015). If a nanoemulsion has percent transmittance greater than 99 %, it is esteemed as transparent in nature.

1.6.13 Conductance measurement

Observation of electrical conductivity is very crucial in assessing the character of the continuous phase, and in detecting phase inversion phenomena. A conductometer is used in this process. An electrical source is submerged in the emulsion in question and two electrodes are connected with a lamp. If the emulsion is o/w type, the lamp will lit up because water from the continuous phase will aid the passage of electricity between the electrodes. No such phenomena will occur in case of w/o emulsions (Jaiswal et al., 2015).

Chapter 2

Methodology

2.1 Materials

Aceclofenac was the active pharmaceutical ingredient (API) used in the nanoemulsion formulations. It was a kind gift from Quality Pharmaceuticals Ltd. Castor oil was used for the oil phase, which was purchased from local general store. The manufacturer is Well's from Madrid-Spain. Tween-80 was used as surfactant. Ethanol was used as co-surfactant. Both were imported from Indonesia. At last, distilled water used for the water phase was prepared in the phytochemistry lab of BRAC University.

2.2 Method

Aceclofenac equivalent of 1.5% of the total formulation was dissolved in the oil phase. API and oil were mixed using a vortex mixer for 15 minutes. Then the mixture was heated in water bath at 50-57°C. A separate mixture of the surfactant and cosurfactant was prepared. This was called 'Smix'. Once the API was dissolved in the oil, the Smix was added to the API-oil mixture and stirred properly. Total 5 pairs of formulations were made, each pair with varying surfactant – co-surfactant percentage. Two techniques were used to mix the oil phase and water phase : homogenization and ultrasonication. The oil phase was slowly poured into the water phase.

The following table consists of the percentage of reagents used in 40 ml formulations where F1, F2, F3, F4 and F5 formulations are prepared using magnetic stirring technique whereas F1′, F2′, F3′, F4′ and F5′ formulations are prepared using ultrasonication technique.

Table 1 : Nanoemulsion formulations

Reagents	F1	F1′	F2	F2′	F3	F3′	F4	F4′	F5	F5′
API	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%
Castor Oil	21.25%	21.25%	21.25%	21.25%	21.25%	21.25%	21.25%	21.25%	21.25%	21.25%
Tween 80	52.5%	52.5%	55%	55%	57.5%	57.5%	60%	60%	62.5%	62.5%
Ethanol	17.7%	17.7%	15.2%	15.2%	12.7%	12.7%	10.2%	10.2%	7.7%	7.7%
Water	7.05%	7.05%	7.05%	7.05%	7.05%	7.05%	7.05%	7.05%	7.05%	7.05%

2.2.1 Physical Stability Study

The formulations were kept in transparent glass containers and kept in room temperature. These were observed for stability.

2.2.2 Microscopic Study

Very small amount of the formulations were taken in slides and dyed with methyl red, which is a water soluble dye. Then the slides were observed under microscope.

2.2.3 Dilution Test

The formulations were diluted 10 times with distilled water and 0.1N HCL and observed.

2.2.4 Conductivity Test

The conductivity of the formulations were measured using a conductivity meter.

2.2.5 Fluoroscence Test

All 10 formulations were observed under UV light.

Chapter 3

Result and Discussion

3.1 Result

3.1.1 Physical stability

The formulations were observed visually after preparation. All of the formulations presented a thick, transparent, golden appearance. The formulations that were prepared by magnetic stirring were relatively thicker than those prepared by ultrasonication. None of the formulations showed phase separation.

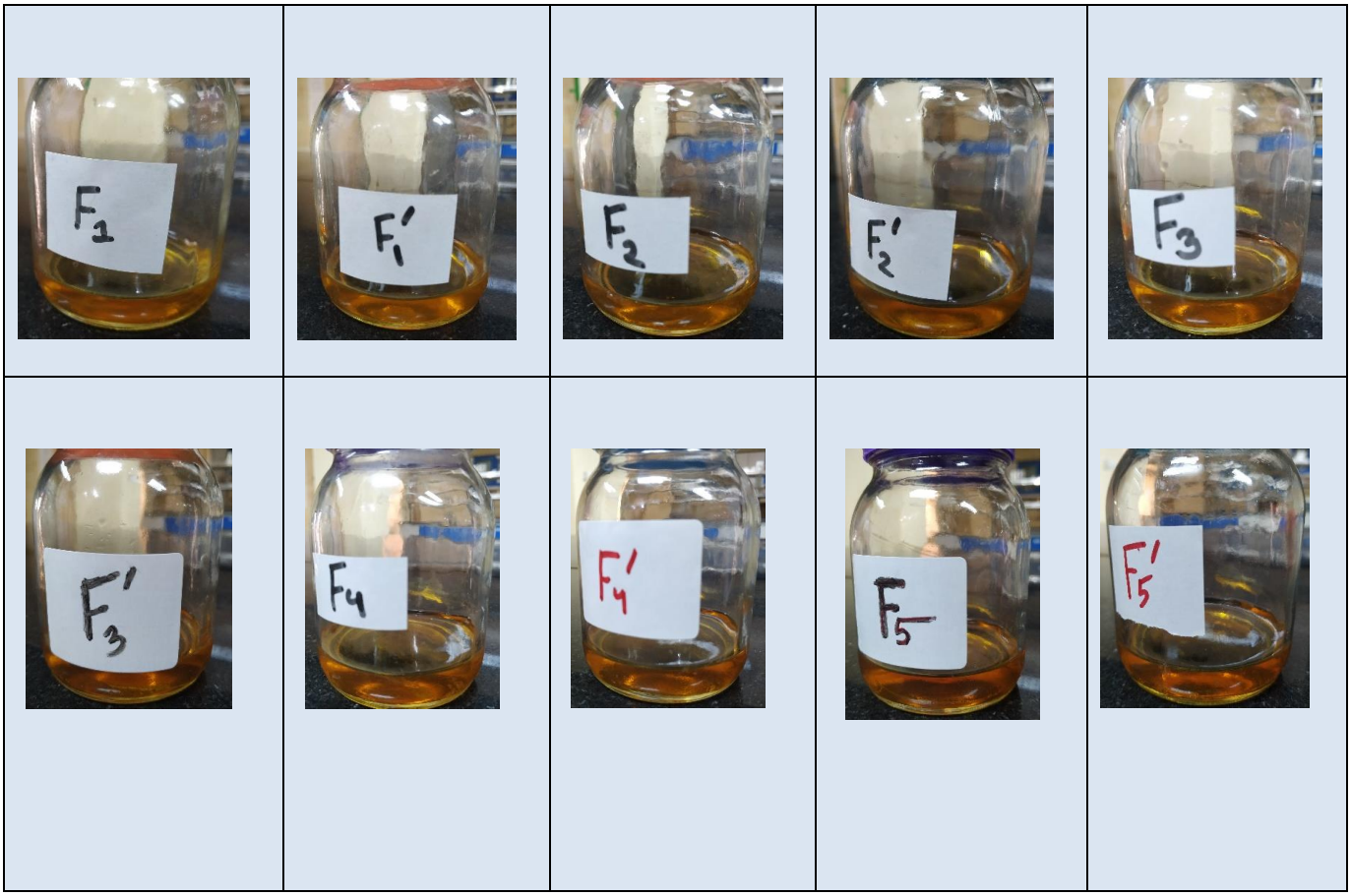


Figure 1 : Aceclofenac nanoemulsions formulations

Observation results are shown in table 2.

Table 2 : Obsrvation of the physical stability of the nanoemulsions after 14 days

Formulation	**Observation**
F1	No phase separation
F1′	No phase separation
F2	No phase separation
F2′	No phase separation
F3	No phase separation
F3′	No phase separation
F4	No phase separation
F4′	No phase separation
F5	No phase separation
F5′	No phase separation

3.1.2 Microscopic Test

The microscopic study of all 10 formulations showed clear image of dyed water globules which appeared red. The number of droplets in all 10 formulations are promising.

The microscopic images are shown in Figure 2.

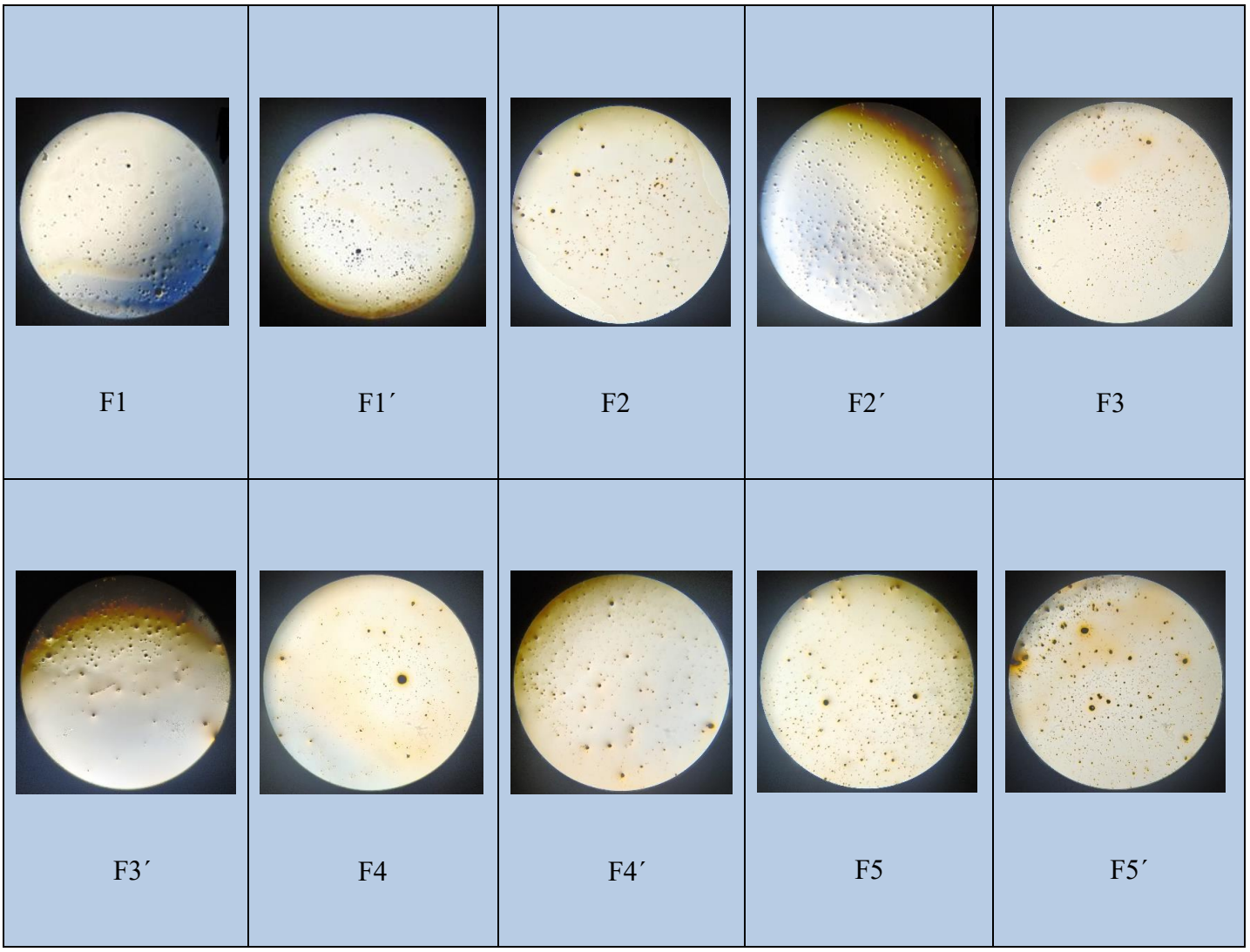


Figure 2 : Microscopic observation of the nanoemulsions

3.1.3 Dilution Test

- Dilution with distilled water :

The formulations were diluted 10 times with distilled water and labelled as F1W, F1′W, F2W, F2′W, F3W, F3′W, F4W, F4′W, F5W and F5′W (Figure 3).

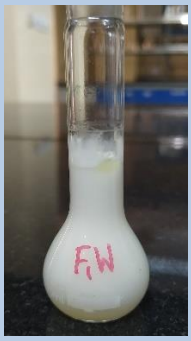
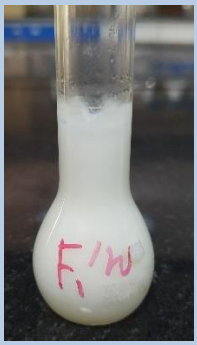
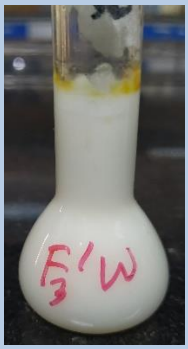
				
F1W	F1′W	F2W	F2′W	F3W
				
F3′W	F4W	F4′W	F5W	F5′W

Figure 3 : Dilution test with distilled water

After dilution, F1′W, F2W, F3W, F3′W, F4′W and F5′W appeared turbid but no phase operation had occurred. F1W, F2′W, F4W and F5W had separated phases.

Table 3 : Observation of dilution test with distilled water after 1 days

Formulation	**Observation**
F1W	Phase separation
F1′W	Turbid but no phase separation
F2W	Turbid but no phase separation
F2′W	Phase separation
F3W	Turbid but no phase separation
F3′W	Turbid but no phase separation
F4W	Phase separation
F4′W	Turbid but no phase separation
F5W	Phase separation
F5′W	Turbid but no phase separation

- Dilution with 0.1N HCl :

The formulations were diluted 10 times with 0.1N HCl and labelled as F1H, F1´H, F2H, F2´H, F3H, F3´H, F4H, F4´H, F5H and F5´H (Figure 4).

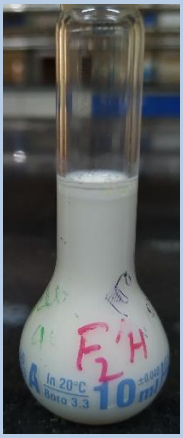
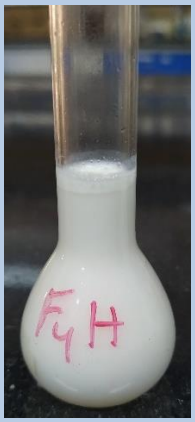
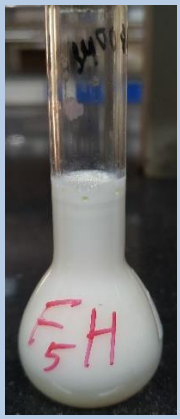
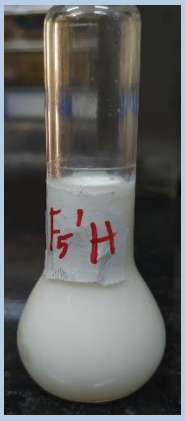
				
F1H	F1´H	F2H	F2´H	F3H
				
F3´H	F4H	F4´H	F5H	F5´H

Figure 4 : Dilution test with 0.1N HCl

After dilution with 0.1N HCl, F4′H and F5H exhibited distinct phase separation whereas F1H, F1′H, F2H, F2′H, F3H, F3′H, F4H and F5′H appeared turbid.

Table 4 : Observation of dilution with 0.1N HCl

Formulation	**Observation**
F1H	Turbid but no phase separation
F1′H	Turbid but no phase separation
F2H	Turbid but no phase separation
F2′H	Turbid but no phase separation
F3H	Turbid but no phase separation
F3′H	Turbid but no phase separation
F4H	Turbid but no phase separation
F4′H	Phase separation
F5H	Phase separation
F5′H	Turbid but no phase separation

3.1.4 Conductivity Test

The electrode of the conductivity meter was dipped in the formulations and the conductivity was measured. The following table shows the conductivity of the formulations.

Table 5 : Conductivity of the formulations

Formulation	**Conductivity**	**% of Ethanol**
F1	8.1	17.7%
F1′	7.5	17.7%
F2	6.8	15.2%
F2′	6.0	15.2%
F3	5.1	12.7%
F3′	6.7	12.7%
F4	5.0	10.2%
F4′	4.4	10.2%
F5	4.5	7.7%
F5′	3.5	7.7%

The decline in conductivity (with the exception of F3′ and F5) can be associated with the gradual decrease in amount of ethanol present in the formulations. This is because ethanol conducts electricity.

The following graph correlates this data :

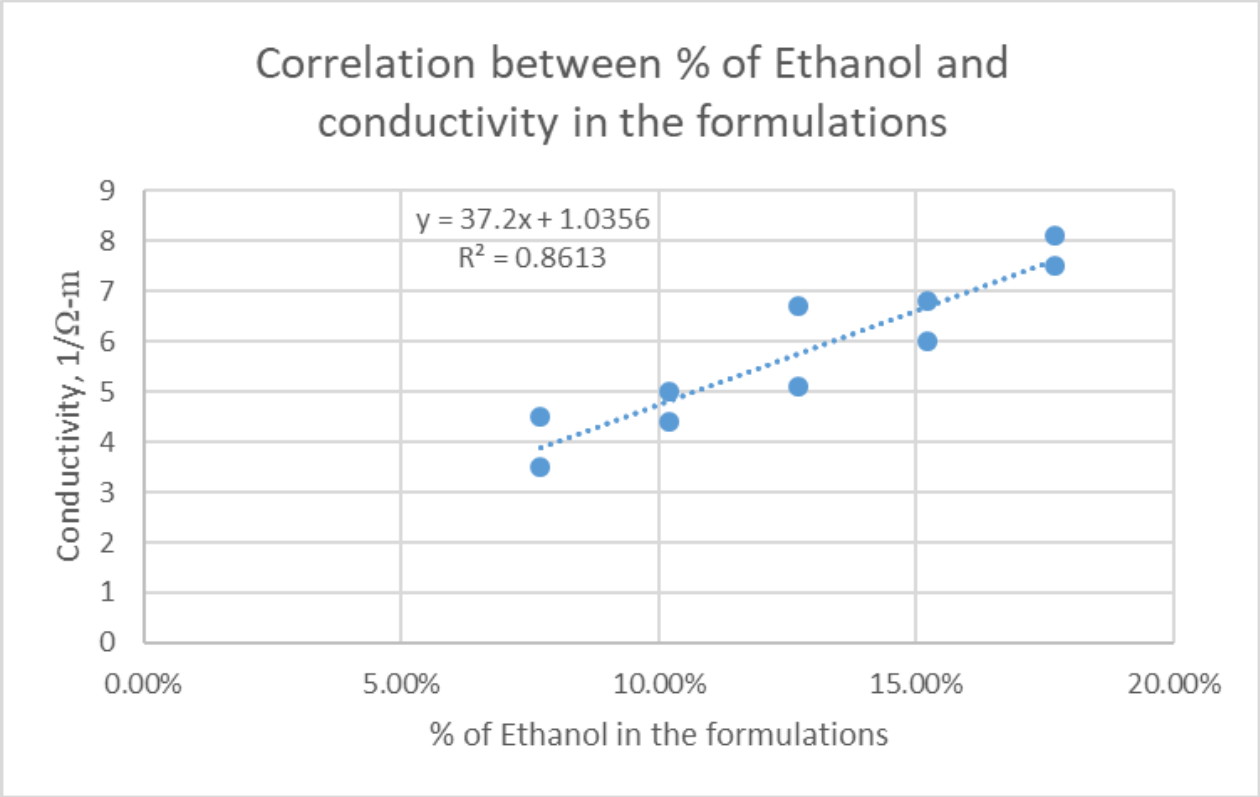


Figure 5 : Correlation graph of % of ethanol and conductivity in the formulations

The R^2 value (0.8613) indicates that there is a strong correlation between % of ethanol in the formulations with their decline in conductivity.

3.1.5 Fluoroscence Test

A small amount of each nanoemulsion formulations were taken in separate petri dishes and observed under Ultraviolet light. All of the formulations exhibited fluorescence (Figure 6), which is an indication for W/O emulsions.

Figure 6 : Fluoroscence test of the nanoemulsions

3.2 Discussion

All 10 formulations should be considered as O/W nanoemulsions on the basis of their preparation technique. The formulations show conductivity, which is an indicating factor for O/W emulsions. On the other hand, the formulations also exhibit fluorescence, which is commonly observed in W/O emulsions. This can be explained by discussing the percentage of oil and water used to prepare the formulations. The percentage of castor oil in these formulations is 21.25%, whereas the percentage of water is 7.05%, which is one-third of oil. It is highly probable that the significantly smaller amount of water in the formulations causes the water to disperse in the oil phase instead of the opposite even though the oil phase was added to the water. The microscopic test showing dyed water globules confirms this hypothesis.

Chapter 4

Conclusion

10 nanoemulsions formulations were prepared, 5 using homogenization and 5 using ultrasonication. The formulations prepared by homogenization appeared thicker than the one prepared by ultrasonication. All formulations were evaluated by performing physical stability study, dye test, microscopic test, dilution test, fluorescence test and conductivity test. Finally, all of the formulations showed stability after 14 days, with the formulations prepared with ultrasonication method showing better stability in dilution test and higher droplet exhibition in the dye test.

4.1 Future studies

Evaluation parameters such as polydispersity index, droplet size analysis, percent transmittance, zeta potential test and in-vitro analysis can be further studied to confirm the formulations as nano emulsions.

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