FORMULATION AND CHARACTERIZATION OF IONIC LIQUID IN OIL MICROEMULSIONS

DILRAJDEEP SINGH

CHEMICAL ENGINEERING

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by

Dilrajdeep Singh
18080

Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
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CERTIFICATION OF APPROVAL

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Approved by,

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January 2017

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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DILRAJDEEP SINGH

ABSTRACT

Recently there have been various breakthroughs in the development of new types of drugs and drug delivery methods. Transdermal drug delivery (TDD) provides a safe and non-invasive method of drug delivery. TDD is yet to reach its full potential due to the poor solubility of many drugs in pharmaceutically accepted solvents. Ionic Liquids (ILs) are known to solubilize that have poor solubility but its application for TDD is limited by the hydrophobic barriers of the skin. This limitation can be overcome by incorporating hydrophobic domains in the IL containing solution by the formation of microemulsions (MEs). This research focusses on the formulation of such MEs by using pharmaceutically accepted components. The IL used in this study is 1-ethyl-3-methylimidazolium acetate. Taguchi's experimental design was used to identify the effects surfactant ratio, surfactant concentration, IL content and temperature towards droplet size and drug solubility. In this approach, nine MEs were formed with varied parameters based on Taguchi's design and ACV was dissolved in these MEs. All the MEs used IPM as the continuous phase and a surfactant blend of Tween-80 and Span-20 was used. It was seen that as surfactant ratio increases (increasing Tween-80), both the droplet size and drug solubility increases. It was also seen that as the surfactant concentration increases there is an increase in drug solubility but there is a decrease in particle size. As IL content and temperature increases there was an increase in both drug solubility and droplet size. The parameter that influences droplet size the most is surfactant ratio whereas the parameter that influences the drug solubility the most is IL content. The parameters were further optimized to identify the conditions that will give the highest drug solubility and lowest droplet size. It was concluded that the optimized conditions can be achieved by using surfactant ratio as 2:3, surfactant concentration as 25wt%, ionic liquid content at 2wt% and temperature at 30°C.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

Abbreviations	Meaning
IL	Ionic Liquid
ME	Microemulsion
O/W	Oil-in-Water
W/O	Water-in-Oil
S/O	Solid in Oil
IL/W ME	Ionic Liquid in Water Microemulsion
IL/O ME	Ionic Liquid in Oil Microemulsion
IPM	Isopropyl myristate
Tween-80	Polyoxyethylene Sorbitan Monooleate
Span-20	Sorbitan Laurate
wt%	Weight Percent
TDD	Transdermal Drug Delivery
PEO	Polyoxyethylene
ACV	Acyclovir
MTX	Methotrexate
DMSO	Dimethyl Sulfoxide
DMF	N,N-Dimethylformamide
OVA	Ovalbumin
API	Active Pharmaceutical Ingredient
DOE	Design of Experiment
SN	Sound to Noise Ratio
DOF	Degree of Freedom
DLS	Dynamic Light Scattering
$[C_2mim][CH_3COO]$	1-Ethyl-3-Methylimidazolium Acetate
$[C_2mim][BF_4]$	1-Ethyl-3-Methylimidazolium Tetrafluoroborate
$[C_2mim][HOSO_3]$	1-Ethyl-3-Methylimidazolium Hydrogensulfate
$[C_2mim][PF_6]$	1-Ethyl-3-Methylimidazolium Hexafluorophosphate

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Pharmaceutical drugs have become one of the most important aspect of the modern world. New drugs are being developed each day to help fight against diseases and illnesses. One of the most important process that relates to drugs is the administration/delivery of the drugs. Drug administration is defined as the method that a person uses to take their medicine. This refers to the technology, methods and systems to transport a drug safely into the body to reach its desired therapeutic effect. The technologies behind delivery can change the drug release profile, distribution and adsorption for increasing the efficacy of product and to improve patient compliance. Currently the most common ways of drug delivery are by using the oral route, topical, hypodermic injections, transdermal and inhalation (Finkel et al., 2009).

Transdermal drug delivery (TDD) suggests a safer and non-invasive method of drug delivery that someday can substitute hypodermic injections and oral delivery. Compared to hypodermic injections and oral delivery, transdermal drug delivery has several advantages. Transdermal drug delivery has the potential to improve patient compliance, it reduces the risk of disease spreads that occurs through needle reuse, and it can prevent first pass hepatic elimination. (Williams, 2003). Over the past several years, numerous approaches for transdermal drug delivery has been created like sonophoresis, iontophoresis and via usage of chemical/lipid enhancers (Prausnitz, 2004). Despite all these developments in technology, transdermal drug delivery is still not at its maximum potential for drug delivery. This is because many drugs are not/sparingly soluble in water and pharmaceutically accepted solvents.

Many of these promising drugs do not enter the formulation stage due to their difficulties in delivery and poor solubility. The sparingly soluble nature of the drugs leads to poor drug bioavailability and absorption through all method of drug delivery including transdermal drug delivery. To add to this problem, several substrates that are used for synthesizing important drugs for example nucleoside analogs, have poor solubility in conventional solvents (Fujita et al., 2005).

One of the drugs that is yet to realize its maximum potential due to poor solubility is Acyclovir(ACV). This drug is mainly used as an antiviral drug to slow the growth rate of herpes virus. ACV is used to treat genital herpes, shingles, chicken pox and cold sores which are infections caused by the herpes virus. Currently, ACV is delivered by using topical creams, tablets ophthalmic ointment and injections. ACV has a low bioavailability (15 – 25%) and the injections are hurtful and will produce hazardous medical waste (Moniruzzaman et al., 2010a).

To tackle the drug solubility problem in the pharmaceutical industry, many new strategies extending from tailoring the drug delivery methods to formulation of drug have been developed. For example, sparingly soluble drugs such as ACV can be dissolved in acidic or basic salts such as hydrochloride(acidic) and sodium(basic). However, these drugs can precipitate when in the body due to pH conditions (Ansel et al., 1999). Another method to dissolve sparingly soluble drugs is the usage of excipients like ethanol and dimethyl sulfoxide (DMSO) together with specific surfactants (Yalkowaky, 1981). Unfortunately, most of the time a significant increase in the solubility only occurs at high concentrations of the excipients and this may lead to unwanted side effects to the human body (Ray et al., 2003). Other than DMSO, the polar organic solvent excipients which are commonly used to dissolve drug with poor solubility are N,N-dimethylformamide (DMF) and pyridine. Although, there organic solvents are flammable, have high toxicity and volatile which makes them not suitable to be used as pharmaceutical ingredients (Yalkowaky, 1981). Most of the time, when these polar solvents are used in drug formulation reaction it produces a huge amount of waste and poses problems in waste disposal due to complications in reuse steps and recovery.

The researches done above clearly states that there is a need to find a more superior and greener solvent to dissolve sparingly soluble drugs. Ionic Liquids (ILs) has shown promising potential as a type of solvent that can overcome the limitations faced by the solvents mentioned above. Recently, Ionic Liquids have been used more and more as solvents, co-solvents and materials in various industries, including the pharmaceutical industry (Anderson, 2003; Jaitely et al., 2008). The application of ILs also extends to the synthesis of active pharmaceutical ingredients (APIs) that has modified solubility, higher thermal stability, and an enhancement in the efficacy of topical analgesia when compared to their initial materials (Hough and Rogers, 2007; Stoimenovski et al., 2010). ILs represent thermally stable, non-volatile, non-flammable and tunable designer solvents that hopefully can replace the highly volatile organic solvents for a wide range of applications (Seddon, 1997; Welton, 1999; Earle and Seddon, 2000; Moniruzzaman et al., 2010b,c). Ionic Liquids can be synthesized with different polarities and/or properties simply by changing the anion/cation combination (Brennecke and Maginn, 2001).

This interesting property of Ionic Liquids enables the dissolution of many sparingly soluble substrates, which leads to the concept of tailor-made solvents for a specific application. Although Ionic Liquids have been used extensively in a wide range of fields, their use in pharmaceutics application is very limited due to the question whether Ionic Liquids high toxicity (Constable et al., 2007). However, considering the tunability of Ionic Liquids, nontoxic Ionic Liquids can be designed by using nontoxic, biodegradable and pharmaceutically acceptable organic cations and inorganic anions. Some biocompatible Ionic Liquids have already been synthesized (Pernak et al., 2004; Weaver et al., 2010).

Fortunately, a few researches have demonstrated low toxicity levels of certain Ionic Liquids towards Caco-2 cells (Jaitely et al., 2008) and female Wistar rats (Pernak et al., 2004). Many beneficial and essential chemicals have toxic properties. Numerous pharmaceutical excipients like DMSO and non-ionic surfactants like polysorbate 80 have shown toxicities that are like what was seen in many Ionic Liquids. Therefore, the toxicity levels of Ionic Liquids do not impede their usage as pharmaceutical solvents.

As discussed above, many sparingly soluble drugs can be solubilized in ILs. However, IL-borne drugs cannot penetrate the skin because of its hydrophobic barrier function, which is provided by the highly organized structure of the stratum corneum. The skin, a natural shield of the body, acts as a barrier against the excessive loss of water and other essential elements. This largest organ of the human body acts as a promising gateway for drug entry with local and/or systemic action. The improved drug concentration in targeted skin layers or in the bloodstream is beneficial during transdermal drug delivery (Patel et al., 2012). The use of prodrugs and penetration enhancers can enhance the transdermal permeability of drugs (Prausnitz, 2004). Microemulsions (MEs) are defined as stable, self-assembling systems of a surfactant or mixture of surfactants in two immiscible fluids (Fanun, 2009). They are well recognized as potential drug carriers due to their nanometre size, stability and biocompatibility. MEs have been widely formulated and implemented for drug delivery across the skin barrier (Lawrence et al., 2000).

To obtain a more statistically strong and accurate data, the Taguchi's Method is used as a design of experiment. The Taguchi's Method is an efficient method of analyzing effects of multiple variables simultaneously. This method incorporates statistics together with robust engineering methods to achieve accelerated improvements in the manufacturing process of products (Kracker,1991). This standardized version of Design of Experiment (DOE) is one of the most effective quality building tools used by engineers in all type of manufacturing activities. By using this statistical model introduced by Dr Genichi Taguchi, we will be able to understand various effects of parameters, in this case; temperature, surfactant concentration, surfactant ratio and Ionic Liquid contend on obtaining an optimum matrix of combination ionic liquid in oil microemulsion to produce the highest possible solubility and stable microemulsion.

1.2 Problem Statement

Transdermal drug delivery is a non-invasive drug delivery method which has yet to achieve its maximum potential due to the low solubility of certain sparingly soluble drugs. Acyclovir is a drug that has very low solubility in water and most organic solvents. An ionic liquid based solvent system could be used to solubilize sparingly soluble drugs such as ACV (Anderson, 2003; Jaitely et al.,2008; Moniruzzaman et al., 2010a). The drugs will be delivered into the body by using microemulsion as the drug carrier system. In previous studies the microemulsion system was formed using an ionic liquid that is not pharmaceutically accepted as they have a toxicity level that is higher than the what is accepted in the pharmaceutical industry (Moniruzzaman et al., 2010a). The end goal of this research is to get the highest drug solubility and highest skin permeation with the usage of low toxic materials. Many studies have shown that high skin permeability is achieved by smaller droplet size. For example, in Diazepam transport the permeability was enhanced by using formulations with dispersed phase below 0.5 μm (Lopes, 2014). This research will focus to tackle the problem below:-

a) Poor solubility of drugs such as ACV in pharmaceutically accepted organic solvents
b) Ionic Liquids used in previous studies have high toxicity and is not pharmaceutically accepted
c) The effects of parameters such as surfactant ratio, surfactant concentration, ionic liquid content towards droplet size and drug solubility of Ionic Liquid in Oil Microemulsion (IL/O ME) is yet to be determined.
d) The optimized parameters that gives the smallest droplet size and highest solubility is rarely investigated.

1.3 Objectives

This research focuses on formulating and characterizing an ionic liquid in oil microemulsion to deliver sparingly soluble drugs. The objectives of this research are as below:

a) To select, form and characterize an Ionic Liquid that has low toxicity and can dissolve sparingly soluble drugs such as ACV.
b) To form and characterize stable Ionic Liquid in Oil Microemulsions.
c) To identify and study effects of the four main factors that influence the drug solubility and droplet size of IL/O ME.
d) To conduct a statistical study on the significance of each parameter towards drug solubility and droplet size.
e) To optimize the parameters that will give the lowest droplet size and highest drug solubility

1.4 Scope of Study

This study will firstly cover the scope for Ionic Liquid preparation. The ionic liquids will be first screened through literature review and research. ILs that have coordinating anions will be preferred in this screening process as it has a higher tendency to dissolve a higher amount of drugs. This study also cover the formulation of microemulsions with different parameters and the analysis of the microemulsions using dynamic light scattering and uv spectrophotometer.

CHAPTER 2

LITERATURE REVIEW

2.1 Ionic Liquids for Solubility of Drugs

Based on recent studies it was observed that an ionic liquid based solvent system can be used to dissolve sparingly soluble drugs (Anderson, 2003; Jaitely et al., 2008; Mizuuchi et al.,2008; Moniruzzaman et al., 2010a). Other than that the ionic liquids can also be used in synthesis of active pharmaceutical ingredients (APIs) with manipulated solubility, higher thermal stability and a notable enhancement in the efficacy of topical analgesia comparing to the initial material. (Hough and Rogers, 2007; Stoimenovski et al., 2010). Ionic liquids with various polarities can be synthesized by manipulating the anion/cation combination (Brennecke and Maginn, 2001). Ionic liquids are thermally stable, non-volatile, tunable and non-flammable solvents that can potentially replace the more volatile organic solvent in a variety of applications (Seddon, 1997; Welton, 1999).

The usage of ionic liquids in the pharmaceutical industry is currently very minimal as there is a risk that ionic liquids have high toxicity which are pharmaceutically unaccepted. (Constable et al., 2007). Given the fact that the ionic liquids are highly tunable, it can be designed to be pharmaceutically accepted by the usage of biodegradable, nontoxic and pharmaceutical grade organic cations and inorganic anions. (Pernak et al., 2004; Weaver et al.,2010). Fig. 2.1 and Fig. 2.2 below shows some commonly used cations and anions of ionic liquids.

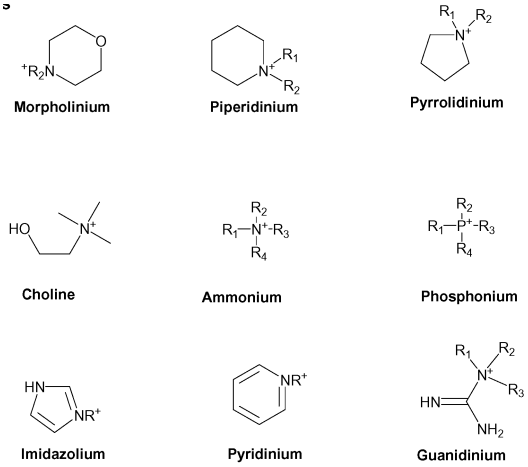

Figure 2.1: Commonly used cations in Ionic Liquids

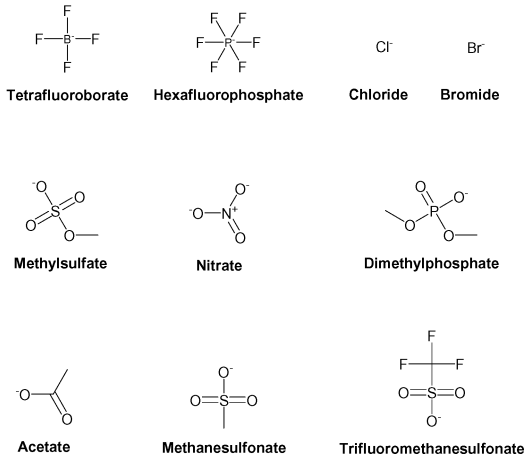

Figure 2.2: Commonly used anions in Ionic Liquids

Recent studies show that hydrophilic ionic liquids that have coordinating anions are effective in the solubilizing ACV, a drug that is insoluble in water and has very limited solubility in organic solvents that are pharmaceutical grade (Moniruzzaman et al., 2010a). Based on the study done by Moniruzzaman et al., 2010a the method of drug delivery using an ACV-IL formulation was not suitable. It was determined that an ionic liquid in oil microemulsion (IL/O) was a suitable method to solubilize the ACV. Building up on this research, the mechanism of formation and encapsulation efficiency of drugs was studied. (Moniruzzaman et al., 2010c). Based on the studies it was seen that IL/O microemulsions can solubilize more amount of the drugs than in their individual components and water. Typical microemulsions are homogenous, transparent and thermodynamically stable dispersions of oil and water

which are stabilized using surfactants. They have emerged as a potential system for drug administration mainly due to their biocompatibility, size and straightforward preparation (Narang et al., 2007).

The fact that ionic liquid is a 'green solvent' has been debated upon in many literatures. However, it can simply be concluded that not all the ionic liquids are considered green and not all of them show nontoxic reactions to mammals. A large number of ionic liquids that contain fluorine does release toxic gases such as POF_3 and HF, after the hydrolysis of a few popular anions namely BR_4^- and PF_6^-.

Table 2.1: Cytotoxicity data for various types of ILs (Adawiyah et al., 2016)

Cation	Anion	Alkyl Chain, R	Cell Cytotoxicity, mM			
			HeLa	$CaCo_2$	MCF-7	NlH/3 T3
1-Alkyl-3 methylimidazolium	CH_3SO_4	CH_3	81.24	-	-	-
1-Alkyl-3-ethylimidazolium	$C_2H_5SO_4$	CH_3	44.11	-	-	-
1-Alkyl-3-methylimidazolium	Cl	C_4H_9	28.69	-	-	-
1-Alkyl-3-methylimidazolium	Cl	C_2H_5	-	32.1	-	51.82
1-Alkyl-3-methylimidazolium	L-lac	C_4H_9	-	19.16	-	33.65
1-Alkyl-3-methylimidazolium	PF_6	C_4H_9	13.9	11.5	-	16.65
1-Alkyl-3-methylimidazolium	BF_4	C_4H_9	5.3	11.19	-	11.3
1-Alkyl-3-methylimidazolium	Cl	C_4H_9	-	6.21	-	6.95
1-Alkyl-3-methylimidazolium	BF_4	C_4H_9	22.9	-	-	-
1-Alkyl-3-methylimidazolium	NTf_2	C_8H_{17}	0.19	-	-	-
1-Alkyl-3-methylimidazolium	CH_3COO	C_2H_5	2.86	-	-	-
Choline Chloride	Cl	-	-	-	-	≥10
DMSO	-	-	-	449	25	≥10
DMF	-	-	-	103	-	-
Ethanol	-	-	-	-	-	≥10
DCM	-	-	-	-	-	≥10

2.2 Ionic Liquid in Oil Microemulsion

For the transdermal delivery of sparingly soluble drugs, Moniruzzaman et al., 2010a have reported IL-in-oil microemulsions (IL/o ME) in which tiny IL droplets are stabilized by a surfactant and a co-surfactant in a continuous oil phase (see Fig. 3). In this ME, drug molecules were loaded in the IL droplets, while the continuous oil phase was used to overcome the stratum corneum barrier, a well-known feature for topical and transdermal transport behavior. They studied the solubility of three sparingly soluble drugs, acyclovir (ACV), methotrexate (MTX) and dantrolene sodium, in IL/o ME. The IL-in-oil MEs were prepared with a blend of nontoxic surfactants, i.e., polyoxyethylene sorbitan monooleate (Tween-80) and sorbitan laurate (Span-20), while isopropyl myristate (IPM) was used as the oil phase (Moniruzzaman et al., 2010b). It was found that only hydrophilic ILs can form IL droplets in the oil phase. Among the different hydrophilic ILs tested, IL [Cmim][DMP] with dissolved drug molecules formed very stable droplets in the IPM continuous phase, stabilized by an interfacial film of a mixture of Tween-80 and Span-20. Previous studies suggested that the dissolution of sparingly soluble drug molecules could be attributed to the interactions between the polar groups of the drug molecules and the IL anions via hydrogen bond formation.

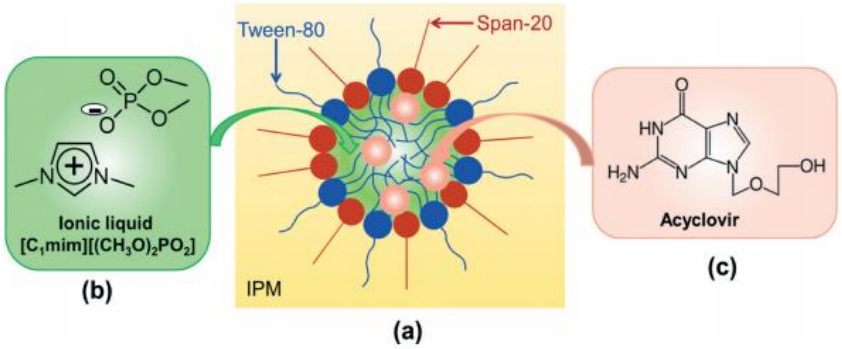

Figure 2.3 Diagram of an Ionic Liquid in Oil Microemulsion (a), Ionic Liquid $[C_1mim][(CH_3O)_2PO_2]$ (b) and ACV Drug (c). Based on Moniruzzaman et al., 2010a

As a potential TDD system, the developed IL/o ME exhibited increased release of the drug ACV in the in vitro evaluation of skin permeability, using full-thickness skin pieces from Yucatan hairless micropigs (YMPs). The enhancement of

ACV penetration by using the IL/o ME was thought to be caused by the high solubility of ACV in the IL dispersion and the effect of the lipophilic components (Moniruzzaman et al., 2010b).

A similar finding of the versatility of IL/o ME as an efficient dermal drug delivery system for sparingly soluble drugs was reported (Yoshiura et al., 2013) in which Methotrexate (MTX) was used as the model drug. IL/o ME was shown to enhance the administration of MTX. Though ILs in their pure form are not biocompatible, an ME containing a low concentration of ILs achieved an increase in the cell viability (>80%) of a cancerous cell line, compared with the controls (i.e., Dulbecco's phosphate-buffered saline (D-PBS), water-in-oil (w/o) ME and IPM) (Moniruzzaman et al., 2010c). This trend in biocompatibility is consistent with those of many widely used chemicals in the pharmaceutical industry. For example, DMSO, a well-known chemical enhancer for TDD, is safe when used at low concentration (up to 10%). However, the presence of highly concentrated DMSO significantly increases the system's cytotoxicity (Williams et al., 2004).

Later, the development of an IL/o ME system consisting of IPM, Tween 80, Span 20 and [C_4mim][Br] for the dermal delivery of the poorly permeating drug 5-fluorouracil (5-FU) was reported (Goindi et al., 2014). The solubility of 5-FU was markedly enhanced in IL/o ME compared with w/o ME. The authors checked the in vivo skin permeation of 5-FU through mouse skin. Application of the selected IL/o ME induced the significant permeation of 5-FU. Importantly, IL/o formulations were found to be effective in the treatment of a skin cancer. The evaluation of IL/o MEs against dimethylbenzanthracene (DMBA)/12-Otetradecanoylphorbol-13-acetate (TPA)-induced mice skin carcinogenesis exhibited that the IL/o ME could treat the skin cancer in 4 weeks. However, the aqueous solution, conventional ointment and commercial cream were ineffective in restoring the normal physiology of the skin after 4 weeks of treatment. Interestingly, the common side effects (e.g., irritation and erythema) related to the conventional drug formulations were not noticed. In addition, histopathological studies corroborated that the application of IL/o ME did not cause any anatomical or pathological changes in the skin structure of the mice.

In a related study using ILs as ingredients in topical drug delivery systems, the influence of the ILs [C_6mim][Cl] and [Cmim][PF_6] on the properties and stability of oil-in-water (o/w) and water-in-oil (w/o) emulsions was described (Dobler et al., 2013). In these systems, the water phase was replaced by hydrophilic IL [C_6mim][Cl], whereas hydrophobic IL [C_4mim][PF_6] was employed to replace the oil phase. Both ILs were successfully incorporated into the emulsion structure, resulting in stable formulations. Compared with the emulsion without IL, the droplet size and viscosity of the IL-based formulation were decreased significantly. The IL-based formulation also maintained long-term stability. The emulsions containing ILs also exhibited consistent pH values. Likewise, the skin penetration enhancement of the fluorescent dyes sodium fluorescein (a hydrophilic fluorescence marker) and Nile red (lipophilic) in the presence of ILs revealed the formulation's more efficient penetration into the deeper skin layers.

Recently, Goindi et al. reported an IL-in-water microemulsion (IL/w ME) formulation that could solubilize Etodolac (ETO), a poorly water-soluble inflammatory drug. The formulation consisted of the IL [C_4mim] [PF_6], while Tween 80 and ethanol were used as surfactant and co-surfactant, respectively. The IL/w ME efficiently enhanced the solubility and skin-permeating ability of ETO for its topical delivery, showing better skin permeation than the other formulations studied. It's in vivo anti-arthritic and anti-inflammatory activities also illustrated the greater effectiveness of the ETO-loaded IL/w ME in controlling inflammation compared with the viscous oily formulation, o/w ME and commercially formulated ETO. Authors also performed histopathological studies to check the anatomical or pathological changes in the skin and observed no changes.

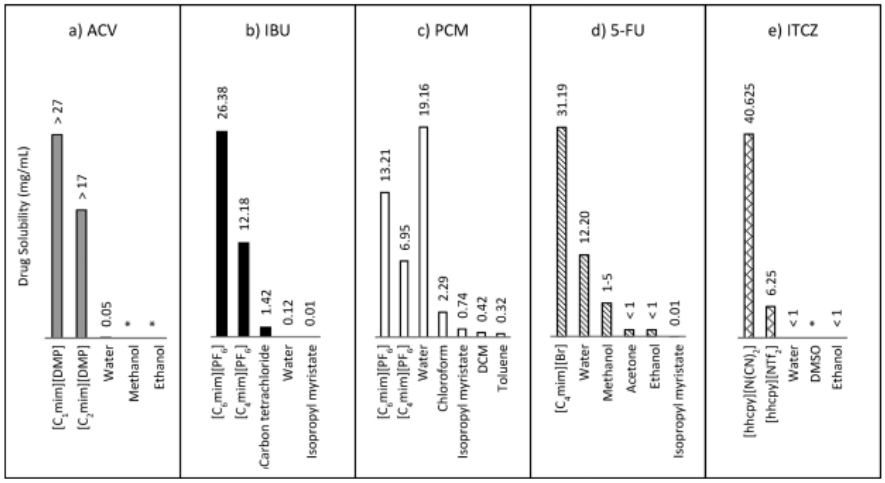

Figure 2.4 Solubility of Drugs in Ionic Liquids Water and Organic Solvents[e]. a) Acyclovir[a], b) ibuprofen[b], c) Paracetamol[b], d) 5-Fluorouracil[c], e) Itraconazole[d].

[a] Moniruzzaman et al., 2010b.

[b] Smith et al., 2011.

[c] Goindi et al., 2014.

[d] Williams et al., 2014.

[e] Jouyban, 2010.

[*] Insoluble

In many of the applications of IL MEs, the use of neat ILs for TDD in the context of drug resistance has been reported. Zakrewsky et al. tested a number of ILs for the enhancement of antibiotic delivery across skin layers. Among the ILs examined, [Chol][Geranate] effectively enhanced permeation for drug delivery. This IL increased the delivery of the antibiotic cefadroxil into the deep tissue layers of the skin by a factor of >16 without inducing skin irritation, while showing minimal toxicity toward several human cancer cell lines. Further supporting its in vivo efficacy, the IL [Chol][Geranate] showed excellent antimicrobial activity against the biofilm protected microbes *Pseudomonas aeruginosa* and *Salmonella enterica*, with >95% bacterial death after 2 h treatment in a biofilm-infected wound model. Continuing the improvement of the dermal delivery of drugs using ILs, Araki et al. recently reported the transcutaneous delivery of a protein consisting of solid-in-oil (S/O) nanodispersions. This study was highlighted on the cover page of Medicinal Chemistry Communications. Here, the IL $[C_{12}mim][NTf_2]$ was introduced as a penetration enhancer in the studied vaccine formulation, resulting in the significantly enhanced skin permeating ability of ovalbumin (OVA), a model antigen. The IL-mediated S/O nanodispersion was also found to elicit high levels of OVA-specific serum IgG (immunoglobulin G) compared with both the S/O nanodispersions lacking the IL and the PBS control. The authors concluded that IL-mediated transcutaneous administration using S/O nanodispersions was effective for high-molecular-weight protein or drug molecules. The study also highlighted the importance of the development of ILs as potential skin penetration enhancers in drug delivery systems.

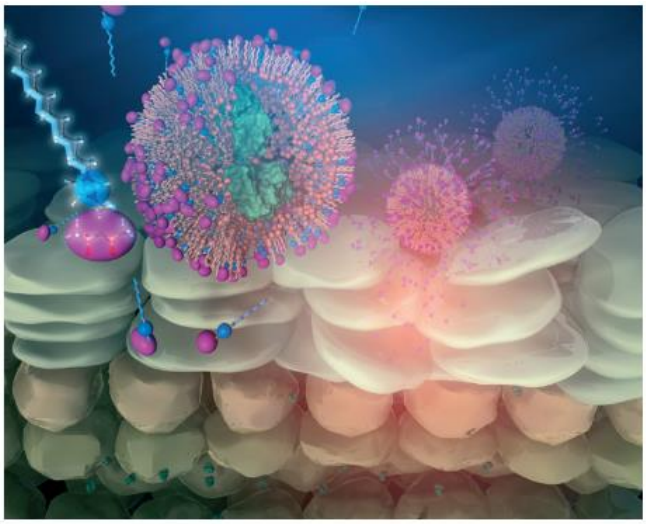

Figure 2.5 Transcutaneous Protein Delivery Consisting of Solid-in-oil (S/O) nanodispersion(Araki et al., 2015)

2.3 Design of experiment

The system of laying out the parameters of investigations including various components was initially proposed by the Englishman, Sir R.A.Fisher. The technique is famously known as the factorial design of tests. A full factorial outline will distinguish every conceivable mix for a given arrangement of components (Kracker,1991). Since most mechanical investigations often include countless, a full factorial plan brings about an expansive number of examinations to lessen the quantity of investigations to a down to practical level, just a little set from every one of the potential outcomes is chosen.

The strategy for selecting a predetermined number of examinations which creates the most data is known as a partial fraction experiment (Bolboaca, 2007). Even though this technique is notable, there are no broad rules for its application or the investigation of the outcomes acquired by the tests. Dr. Genechi Taguchi built a unique arrangement of general plan rules for factorial trials that cover various engineering applications which is known as Taguchi's Method.

Table below shows the comparison of how Taguchi's method will enable reduction of experiment runs while being able to investigate effect of all processing

parameters, in this research there will be four factors and three levels of variations for each factor which substantially reduce the number of experiment to nine times.

Table 2.2 Factorial Design vs Taguchi's Method

Factors	Level	Total number of experiments	
		Factorial Design	Taguchi's Method
2	2	$4(2^2)$	4
3	2	$8(2^3)$	4
4	2	$16(2^4)$	8
7	2	$128(2^7)$	8
15	2	$32{,}768(2^{15})$	16
4	3	$81(3^4)$	9

In order to put into work the Taguchi's method, orthogonal arrays must be used. It provides a set of well balanced (minimum) experiment, serve as objective function for optimization, help in data analysis and prediction of optimum results (Kacker, 1991; Bolboaca, 2007). The effects of many different parameters can be studied in an orthogonal array design.

While there are many standard orthogonal arrays available, each of the arrays is meant for a specific number of independent design variables and levels. Orthogonal arrays are designed in various layout based on the Latin Square format. In this research, three levels of variation were chosen for each parameter as running three levels for the control factors can evaluate non- linearity over the range of control factor compared to two levels which can only evaluate linear effects of factors (Wu, 1986).

The orthogonal layout design can be formed using the following equation:

$$L_n(3^k) \qquad k = (n-1) / 2$$

L = Latin Square layout

n = number of experiments

k = greatest number of factors that can be investigated using the design layout

The minimum number of experiment to be conducted by Taguchi method can be calculated by finding the degree of freedom.

$$N_{Taguchi} = 1 + \sum(Number\ of\ levels\ for\ each\ factor - 1) \quad (1)$$

The $L_9(3^4)$ orthogonal design matrix was picked for this research as it involves four processing parameters while running three levels for each factor and using the degree of freedom approach, nine experiments are needed to understand the effect of all four parameters. It is also crucial to understand the properties of a $L_9(3^4)$ orthogonal design matrix as that would give a further insight on data optimization. As portrayed in Table 3 below, the vertical column shows all setting appears equal in the number of time. For example, under variable four, level 1,2 and 3 appear thrice, this is called the balanced property of orthogonal array (Kacker, 1991).

Table 2.3: L_9 Orthogonal Array Matrix

$L_9(3^4)$ Orthogonal Array					
Experiment #	**Variable 1**	**Variable 2**	**Variable 3**	**Variable 4**	**Performance Parameter Value**
1	1	1	1	1	p1
2	1	2	2	2	p2
3	1	3	3	3	p3
4	2	1	2	3	p4
5	2	2	3	1	p5
6	2	3	1	2	p6
7	3	1	3	2	p7
8	3	2	1	3	p8
9	3	3	2	1	p9

CHAPTER 3

METHODOLOGY

3.1 Project Activities

This research consists of activities like the preparation of material and equipment, laboratory experiment, parameter optimization, process characterization and data analysis. The laboratory experiment includes solubility studies, preparation of surfactant solution and the microemulsion by adding the ILs into the surfactant/IPM solution. The details of the activities are as mentioned in the sequence below.

3.1.1 Materials Preparation

Span-20 (sorbitan laurate) and Tween-80 (polyoxyethylene sorbitan monooleate) was purchased from Sigma–Aldrich Chemical Co. and were of the maximum purity commercially obtainable. These chemicals were used directly without additional purification. All ILs except 1-ethyl-3-methylimodazolium acetate (was synthesized) were obtained from Sigma-Aldrich Chemical Co. and were directly used without any additional purification. Acyclovir was obtained from Centre of Research in Ionic Liquids (CORIL), Universiti Teknologi Petronas. The Isopropyl myristate (IPM) also was bought from Sigma–Aldrich Chemical Co. and was used as received. Water was doubly deionized and distilled. Every other chemical used in the experiments were of analytical grade.

3.1.2 Synthesis of 1-ethyl-3-methylimidazolium acetate

The synthesis of 1-ethyl-3-methylimidazolium acetate was done by using the metathesis reaction between 1-ethyl-3-methylimidazolium chloride (available in laboratory) and Sodium Acetate. 0.1mol of 1-ethyl-3-methylimidazolium chloride solid is added to 0.1mol of sodium acetate powder and it is then dissolved completely in ethanol. The solution is continuously stirred for the next 72 hours at 25^{o}C, allowing for metathesis reaction to take place. The metathesis process is the process in which the cations and anions of two different components exchange partners. The chemistry behind this process occurs in several steps. The chemicals are first dissolved in ethanol and they become hydrated ions as shown below.

$$[C_2mim][Cl]\ (s) \rightarrow [C_2mim]^+ + [Cl]^- \quad (2)$$

$$[Na][CH_3COO] \rightarrow [Na]^+ + [CH_3COO]^- \quad (3)$$

When then sodium cation meets the chloride anion in the solution they combine to form sodium chloride salt which is insoluble in ethanol and will precipitate forming white precipitates. The precipitates are then filtered out by using a filter paper and the filtrate consisting of ethanol and 1-ethyl-3-methylimidazolium acetate is collected in a rotary evaporator flask. The filtrate then goes through rotary evaporation process to remove the solvent ethanol. The remaining solution was then further dissolved in acetone as a precautionary step to ensure all the sodium and chloride ions have been removed from the system. The solution is continuously stirred for the next 72 hours at 25^{o}C, allowing once again for metathesis reaction to take place. After 72 hours, it was observed that a much smaller amount of sodium chloride was precipitated. The remaining sodium chloride precipitates are filtered out using filter paper and the filtrate goes through the rotary evaporator to remove the acetone solvent. The solution left is 1-ethyl-3-methylimidazolium acetate and it appears as a yellowish and clear liquid. The ionic liquid is stored in two different tube, one for solubility study and one for formulation of microemulsion.

Fig. 3.1: Ionic Liquid 1-ethyl-3-methylimidazolium acetate

3.1.3 Selection and Solubility Study of Ionic Liquid

Out of the many cations that are used in synthesis of ionic liquids the imidazolium cations have been proven to dissolve sparingly soluble drugs effectively (Moniruzzaman, 2010a). An initial screening was done to identify 4 imidazolium based ionic liquids that has high tendency to dissolve drugs and have relatively low cytotoxicity. The shortlisted ILs can be seen in table 3.1 below.

Table 3.1: Cell Cytotoxicity Levels of Ionic Liquids

Name of Ionic Liquid	Abbreviation	Cell Cytotoxicity (mM)
1-Ethyl-3-Methylimidazolium Acetate	$[C_2mim][CH_3COO]$	2.86
1-Ethyl-3-Methylimidazolium Tetrafluoroborate	$[C_2mim][BF_4]$	5.3
1-Ethyl-3-Methylimidazolium Hydrogensulfate	$[C_2mim][HOSO_3]$	42.1
1-Ethyl-3-Methylimidazolium Hexafluorophosphate	$[C_2mim][PF_6]$	27.3

To select the best IL that will be used in the development of microemulsion, a solubility test is done to identify the maximum solubility of the ILs in a surfactant mixture and to identify the water miscibility of the ILs. The nonionic surfactants, Tween-80, Span-20 are widely used in O/W or W/O MEs, specifically for the preparation of drug delivery methods (Wang et al., 2009; Tsai et al., 2010). For this research, the above-mentioned surfactants were selected for two main reasons. Firstly,

the Tween-80 has a hydrophilic polyoxyethylene (PEO) groups, which has a robust affinity with the imidazolium cation of the ILs (Gao et al., 2006). Secondly, the combination of Tween-80 and Span-20 can allow varied interfacial properties (Lu, 2000) which can be an important factor for the formation of IL supported MEs. For the continuous phase IPM is selected due to its low toxicity and extensive usage in other type of microemulsions (Heuschkel, 2008).

To conduct the solubility study, the first thing done was to prepare the IPM/Surfactant solution. A mixture of Tween-80 and Span-20 was prepared at a constant weight ratio of 2:3 (Tween-80 : Span-20 = 2:3 (w : w)) and was mixing until a homogeneous mixture was formed. Then IPM was added to form a solution with the surfactant concentration 20wt%, the mixture was stirred using a vortex until the solution appears macroscopically homogeneous. Four different screw capped glass vials were prepared and the 1g of IPM/surfactant solution was added to each vial. The solution in each vial was titrated with respective ILs until it turned cloudy. This was done to determine the maximum solubility of each IL in the IPM/Surfactant solution.

The next step was to identify the water miscibility of each ionic liquid. 0.5 g of each ionic liquid is added to excess water and is stirred for one hour. After one hour, the water miscibility of IL is recorded.

3.1.4 Selection and Preparation of Surfactants

Surfactants are compounds whose function is to lower the surface/interfacial tension between two liquids. In the case of this research the surfactants are needed to act as an agent to stabilize the microemulsion. The surfactant selected should be low in toxicity and pharmaceutically accepted. Two promising candidates are the nonionic surfactants sorbitan laurate (Span-20) and polyoxyethylene sorbitan monooleate (Tween-80). The surfactants along with IPM are often used in oil-in-water and water-in-oil MEs, especially for the preparation of drug delivery systems. The Tween-80 surfactant is very appropriate as it has hydrophilic PEO groups which has robust affinity with the imidazolium cation of the ILs.

The next part after the selection of surfactant is to determine the weight ratio of surfactant. Assume the surfactants selected are called surfactant A and surfactant B respectively. Different ratio of surfactant A and B will be used to make the same amount of surfactant solution. For example, if the amount of solution needed is 5g and the ratio of A to B is chosen to be 3:2 then 3g for surfactant will be added to 2g of surfactant B. The amount of surfactant solution that will be produced for each ratio is yet to be determined based on the supply available.

3.1.5 Taguchi's Experimental Design

The experimental design for this study is Taguchi's method and to use this method an orthogonal array needs to be developed. Further details on this array is provided in Chapter 2 Literature Review (Bolboaca, 2007). The orthogonal layout design can be formed using equation below and the minimum number of experiments can be identified by using equations below.

$$N_{Taguchi} = 1 + \sum(Number\ of\ levels\ for\ each\ factor - 1)$$

$$N_{Taguchi} = 1 + \sum((3-1) + (3-1) + (3-1) + (3-1))$$

$$N_{Taguchi} = 9\ experiments$$

Based on the equation above the $L_9(3^4)$ orthogonal design matrix was picked for this research as it involves four processing parameters while running three levels for each factor and using the degree of freedom approach, nine experiments are needed to understand the effect of all four parameters. Table 3.2 below shows the levels chosen for each parameter. Table 3.3 below is generated by using the orthogonal array method.

Table 3.2: Selected Levels for Taguchi Design

Parameter	Name	Level 1	Level 2	Level 3
P1	Ratio of Tween-80:Span-20	1:2	2:3	2:1
P2 (wt%)	Concentration of Surfactant in IPM	15	20	25
P3 (wt%)	IL Content	1	2	3
P4 (^oC)	Temperature	25	30	35

Table 3.3: Orthogonal Array for Taguchi Design

$L_9(3^4)$ Orthogonal Array						
ME	**P1 Ratio of Tween-80:Span-20**	**P2 Concentration of Surfactant in IPM (wt%)**	**P3 IL Content (wt%)**	**P4 Temperature (ºC)**	**Response**	**Sound to Noise Ratio**
1	1:2	15	1	25	R1	SN1
2	1:2	20	2	30	R2	SN2
3	1:2	25	3	35	R3	SN3
4	2:3	15	2	35	R4	SN4
5	2:3	20	3	25	R5	SN5
6	2:3	25	1	30	R6	SN6
7	2:1	15	3	30	R7	SN7
8	2:1	20	1	35	R8	SN8
9	2:1	25	2	25	R9	SN9

After getting the responses for the respective analysis, the Sound to Noise (SN) Ratio was calculated for each response. There are three methods of calculating depending on the desired performance response (Bolboaca, 2007). These three method as follows.

a) Smaller the better (Make the response as small as possible)

This can be calculated using the equation below.

$$SN_S = -10 \log \left[\frac{1}{n}\sum_{i=1}^{n} y_i^2\right] \quad (4)$$

b) Nominal the best (Reduces variability around target)

This can be calculated using equation below.

$$SN_T = 10 \log \left[\frac{\bar{y}^2}{S^2}\right] \quad (5)$$

c) Larger the better (Make the response as large as possible)

This can be calculated using the equation below.

$$SN_L = -10 \log \left[\frac{1}{n}\sum_{i=1}^{n} \frac{1}{y_i^2}\right] \quad (6)$$

Where y_i = the i^{th} response

n = number of responses

S = variance of y

$\bar{y}$ = mean of y

$$\bar{y} = \frac{1}{n}\sum_{i=1}^{n} y_i \quad (7)$$

$$S = \sqrt{\sum_{i=1}^{n} \frac{(y_i - \bar{y})^2}{n-1}} \quad (8)$$

After calculating the SN ratio, the next step is to calculate the combinational effect every level of each parameter has towards the response. This can be calculated based on the table 3.4 below.

Table 3.4: Combinational Effect of Each Level Towards Response

Parameter	Level 1	Level 2	Level 3
P1	(SN1+SN2+SN3)/3	(SN4+SN5+SN6)/3	(SN7+SN8+SN9)/3
P2	(SN1+SN4+SN7)/3	(SN2+SN5+SN8)/3	(SN3+SN6+SN9)/3
P3	(SN1+SN6+SN8)/3	(SN2+SN4+SN9)/3	(SN3+SN5+SN7)/3
P4	(SN1+SN5+SN9)/3	(SN2+SN6+SN7)/3	(SN3+SN4+SN8)/3

3.1.6 Preparation of Microemulsion

To further study the MEs, samples with IL were prepared as follows. Tween-80/Span-20/IPM systems were prepared as mentioned in Taguchi experimental design. Nine screw capped glass vials were prepared and the IPM/surfactant solution was added based on the ratio and surfactant concentration in the experimental design (Table 3.3). The ionic liquid is then added and the solution is mixed continuously until a macroscopically homogeneous and clear solution was formed. ACV was later added in excess to the MEs and each vial was stirred continuously for 72 hours under the temperatures determined in the experimental design. After the completion of the 72 hours the samples are equilibrated for 24h and no macroscopic heterogeneity was observed before measurements. Samples were filtered through a 0.45 µm Millipore Millex-LG filter to remove any precipitated drugs, traces of dust or contaminants prior to the measurements. For droplet size, measurements were conducted at room temperature in triplicate. Samples were equilibrated for 20 min before data collection. The droplet size and polydispersity of the dispersed ionic liquid phase were evaluated with the aid of Malvern DTS software. The amount of drugs in the resulting clear filtrate was determined using a UV spectrophotometer at 252 nm for acyclovir.

3.1.7 Process Flow Chart

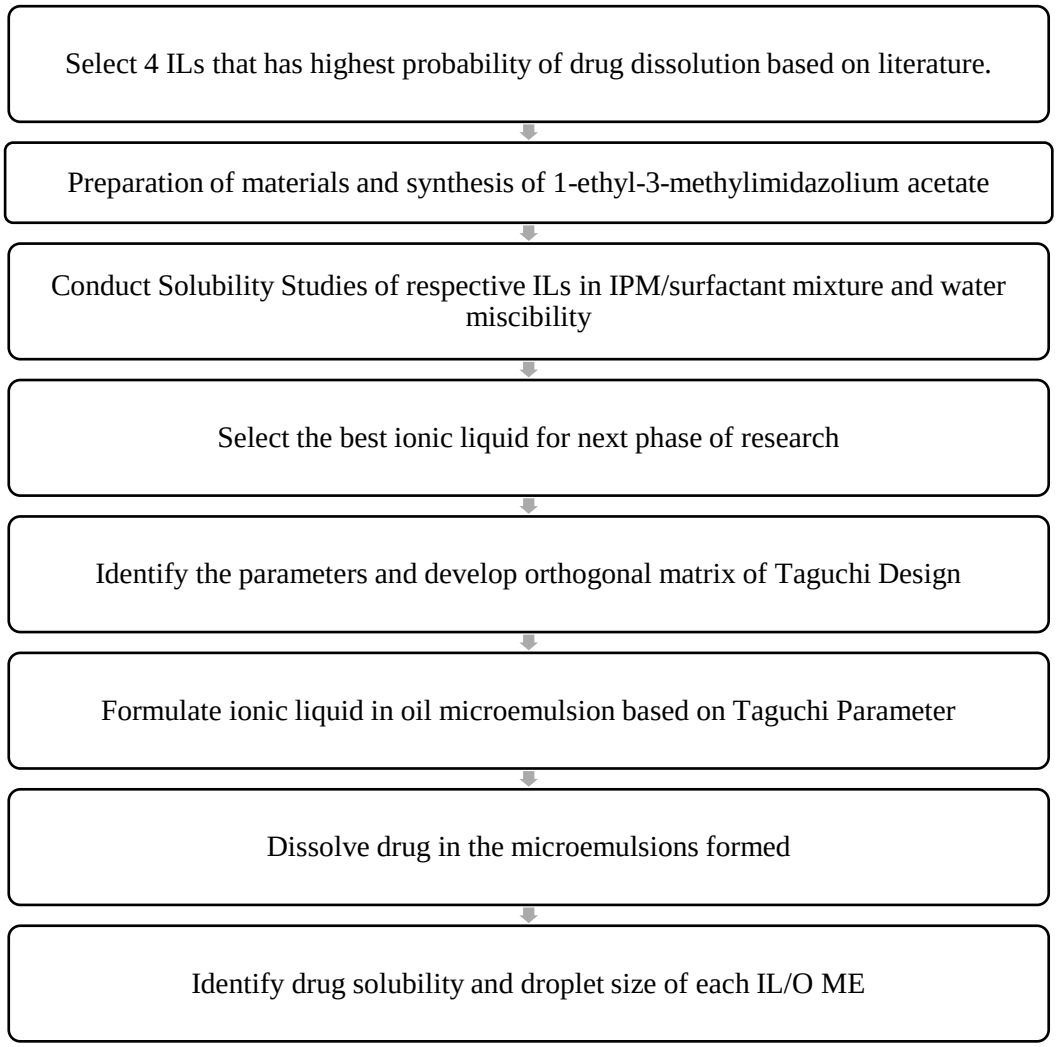

Figure 3.2: Flowchart to Completion of Project

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Solubility of Ionic Liquid in Surfactant/IPM System

The first test was done to investigate the solubility of 1-Ethyl-3-Methylimidazolium Acetate ([C_2mim][CH_3COO^-]) in the oil-surfactant system that was used to create the microemulsion. The solubility test was also done with other Ionic Liquids as mentioned in the methodology section. The ionic liquids are ([C_2mim][BF_4]), ([C_2mim][$HOSO_3$]) and ([C_2mim][PF_6]). As mentioned previously the solvent used for this study is a system with Tween-80 and Span-20 (Tween-80:Span-20 = 2:3(w:w)) stabilized micelles in IPM with 20wt% surfactants. Each one of the ILs was also dissolved in water to observe its miscibility in water. Table below shows the results of the solubility and water miscibility study and the figure below shows the comparison of the maximum solubility of each IL in their respective microemulsion.

Table 4.1: Solubility and Water Miscibility of Ionic Liquids

Name of Ionic Liquid	Abbreviation	Solubility in microemulsion (mg IL / 1g sample)	Water Miscibility
1-Ethyl-3-Methylimidazolium Acetate	[C_2mim][CH_3COO^-]	56.2	Yes
1-Ethyl-3-Methylimidazolium Tetrafluoroborate	[C_2mim][BF_4]	35.3	Yes
1-Ethyl-3-Methylimidazolium Hydrogensulfate	[C_2mim][$HOSO_3$]	42.1	Yes
1-Ethyl-3-Methylimidazolium Hexafluorophosphate	[C_2mim][PF_6]	11.5	No

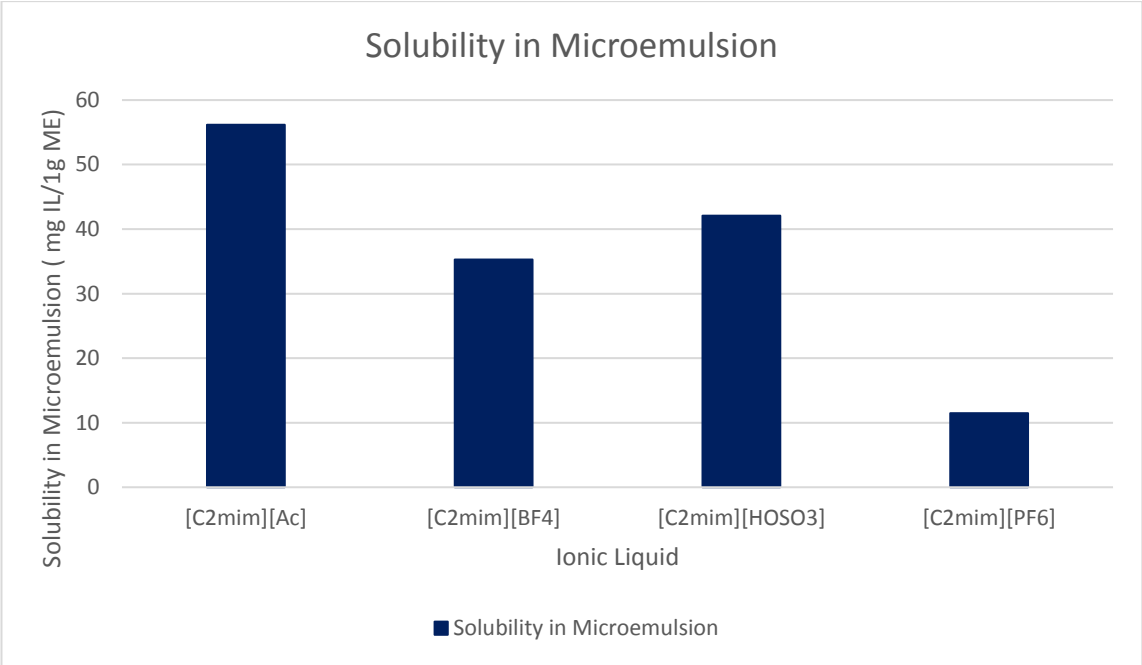

Figure 4.1: Comparison Between IL Solubility in IPM/Surfactant Solution

Based on the results above it can be seen that the ionic liquids which has coordinating anions ($[CH_3COO]^-$ and $[HOSO_3]^-$) can dissolve in the Tween-80/Span-20/IPM core, while ionic liquids that have non-coordinating anions ($[BF_4]^-$ and $[PF_6]^-$) are seen to have poor solubility. The tendency of each IL to dissolve in the ME droplets can partly be explained by considering their solubility in water. Among all the ILs that were studied, IL $[PF_6]$ was observed to have the lowest solubility in water. Figure 4.1 shows that as the alkyl chain length for a similar anionic group increases, the solubility of IL decreases. This occurs because of the increased hydrophobicity of the ILs. A somewhat similar behavior was observed for the solubility of ionic liquids in bulk water (Huddleston et al., 2001). Hence, solubility inside the ME droplet is seen to mirror the solubility of these ILs in bulk water. Furthermore, ILs that have coordinating anions which are strong acceptors of hydrogen bonds (Fukaya et al., 2008) were observed as being extremely suitable when used as a disperse phase in the bulk IPM stabilized by a blend of Tween-80 and Span-20.

Since every ILs utilized as part of the present study is not miscible with IPM, they should be in the hydrophilic micellar core. Both Tween-80 and Span-20 contain hydroxyl groups that has the potential to create hydrogen bonds with the anions of ILs (Fukaya et al., 2008). Multiple hydrogen bonding sites are located on the head groups

of surfactants. Furthermore, Tween-80 has hydrophilic polyoxyethylene oxide (PEO) groups, which has the robust affinity to imidazolium cation attached in ILs via the electrostatic interaction (Gao et al., 2006). The rather impressive solubility of IL [C_2mim][BF_4] in this system shows the presence of electrostatic interactions. This is due to the anion BF_4 usually exhibits poor ability in forming hydrogen bonds with surfactants head groups.

To validate this theory, the solubility of two ILs [C_2mim][CH_3COO] which contains coordinating anions and [C_2mim][BF_4] which contains non-coordinating was tested. For this validation, the Tween-80 was slowly substituted by a similar weight of Span-20 and keeping the surfactant concentration constant. The capacity for solubility was recorded and is shown in figure 4.2.

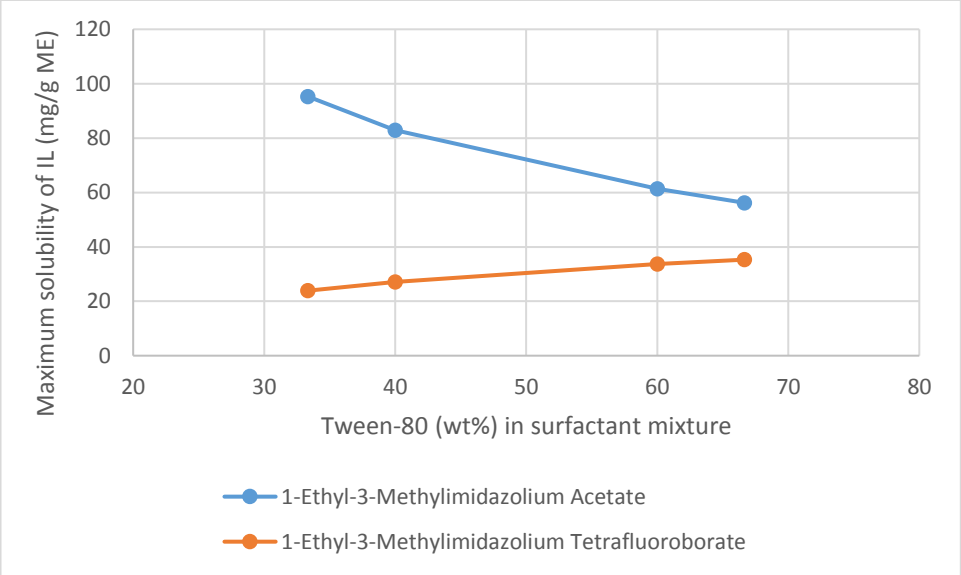

Figure 4.2: Effect of Increasing Tween-80 Content on the Solubility of ILs

Figure 4.2 above shows that solubility of [C_2mim][BF_4] increases when the Tween-80 content increases. The increase in Tween-80 concentration increases the concentration of Tween-80 at the interfacial region. This leads to an increase in number of EO groups which will interact with electropositive imidazolium ring of ILs. Therefore, solubility of this IL is higher at a higher Tween-80 content. Unlike [C_2mim][BF_4] , the solubility of IL [C_2mim][CH_3COO] was seen to decrease with an increase Tween-80 content in the surfactant mixture. However, it was observed that IL with coordinating anion ([C_2mim][CH_3COO]) had greater solubility than IL with non-

coordinating anions ([C_2mim][BF_4]) in any concentration of Tween-80. This superior solubility of [C_2mim][CH_3COO] is due to the dual interactions (electrostatic interaction and hydrogen bonding) of this IL with head groups of surfactants as discussed above. It's possible that both type of interactions is present but the predominance of one or the other rests on the system. Based on the discussions above it can be said that the IL is a glue which bonds the head groups of the surfactant together. This bonding later acts as the driving force to form stable ME droplets in IPM with a blend of Tween-80 and Span-20. Due to IL [C_2mim][CH_3COO] showed a higher solubility and less cytotoxicity compared to the other ILs, it was selected for the next phase of the experiment. Nine different MEs were prepared based on the steps stated in the methodology section and the ternary diagrams below were plotted based on the composition of the MEs.

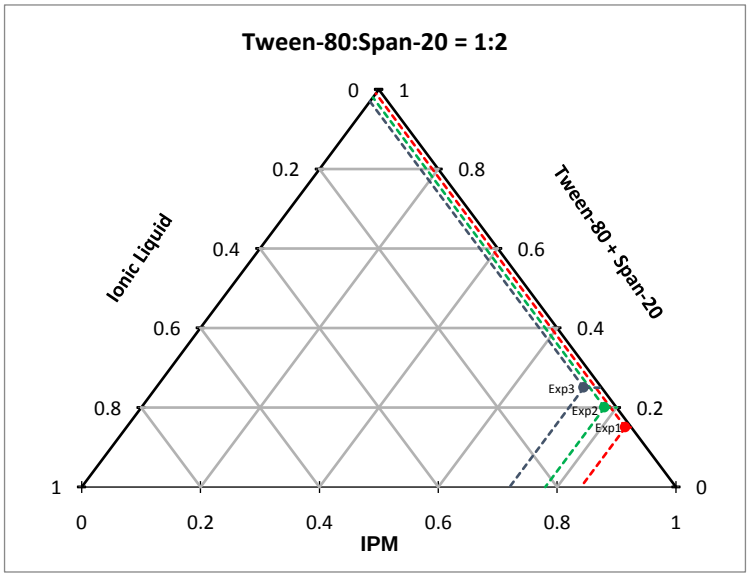

Figure 4.3: Ternary Diagram of [C_2mim][CH_3COO]/Tween-80/Span-20/IPM Four Component System. Weight ratio of Tween-80 to Span-20 is 1:2

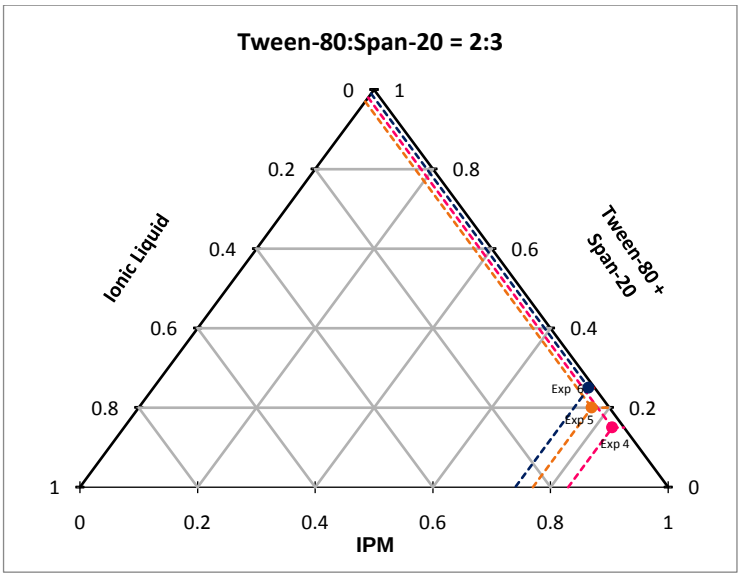

Figure 4.4: Ternary Diagram of $[C_2mim][CH_3COO]$/Tween-80/Span-20/IPM Four Component System. Weight ratio of Tween-80 to Span-20 is 2:3

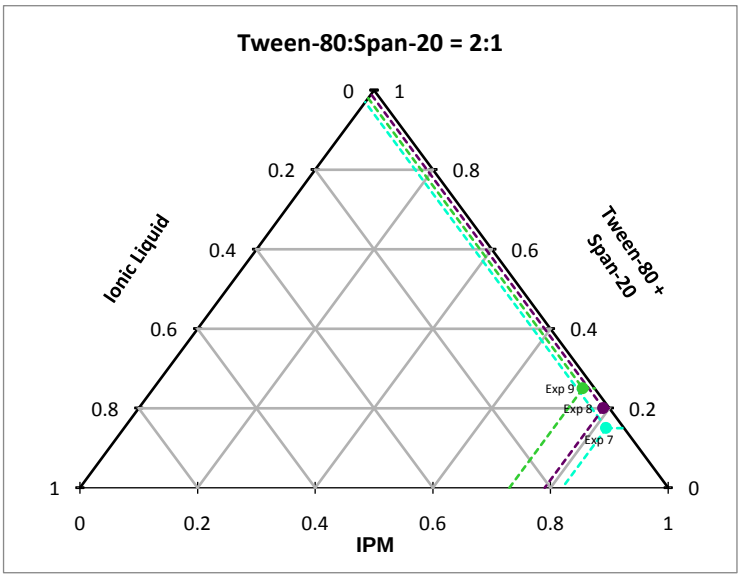

Figure 4.5: Ternary Diagram of $[C_2mim][CH_3COO]$/Tween-80/Span-20/IPM Four Component System. Weight ratio of Tween-80 to Span-20 is 2:1

4.2 Microemulsion Droplet Size Measurement

The shape, sizes and size distribution of droplets in the microemulsion were characterized by dynamic light scattering (DLS). The variation in the sizes of micelles for each experiment was examined and by using Taguchi's experimental design. The results are as shown in the figure 4.6 below.

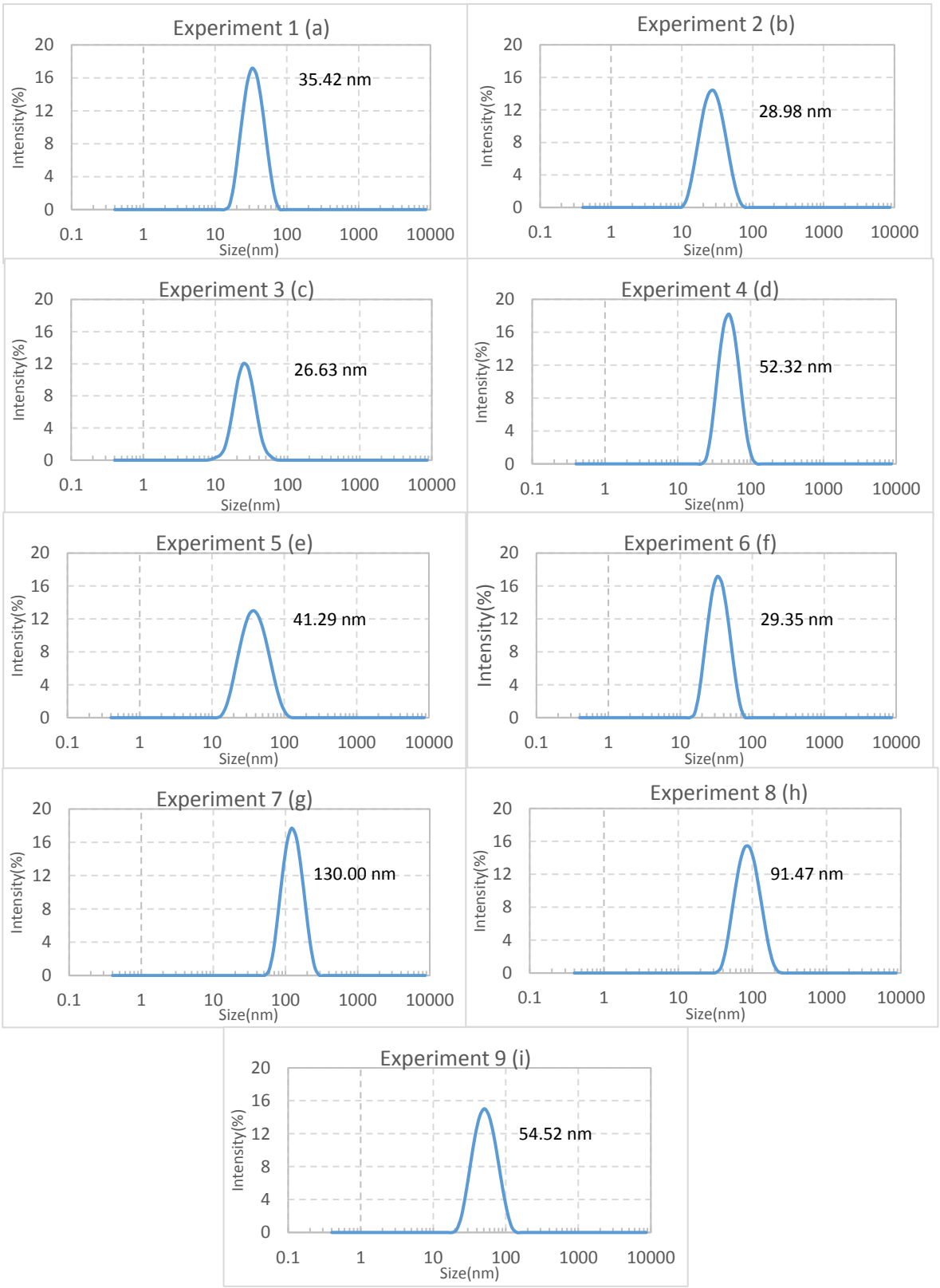

Figure 4.6: Sizes and Size Distribution of Droplets in $[C_2mim][CH_3COO]$/Tween-80/Span-20/IPM Microemulsion with Various Parameters based on Taguchi Design. (a) Experiment 1, (b) Experiment 2, (c) Experiment 3, (d) Experiment 4, (e) Experiment 5, (f) Experiment 6, (g) Experiment 7, (h) Experiment 8, (i) Experiment 9

Table 4.2: Responses and Signal to Noise Ratio for Droplet Size Analysis

ME	Process parameter				Results				
	P_1	P_2 (wt%)	P_3 (wt%)	P_4 (°C)	d_1 (nm)	d_2 (nm)	d_3 (nm)	d_{avg} (nm)	Signal / Noise
1	1:2	15	1	25	36.10	34.73	35.44	35.42	27.30
2	1:2	20	2	30	28.69	29.13	29.12	28.98	29.22
3	1:2	25	3	35	25.76	28.60	25.54	26.63	28.65
4	2:3	15	2	35	53.94	52.07	50.94	52.32	34.48
5	2:3	20	3	25	42.54	41.13	40.19	41.29	32.43
6	2:3	25	1	30	28.35	29.33	30.36	29.35	29.20
7	2:1	15	3	30	131.10	127.80	131.10	130.00	42.24
8	2:1	20	1	35	93.82	90.52	90.06	91.47	39.29
9	2:1	25	2	25	52.26	55.35	55.95	54.52	34.61

Table 4.2 above shows the data obtained on the size of the droplets formed in the various MEs. Each sample was run through the DLS machine three times and the average droplet size was identified. This experimental design demonstrates the effect of each parameter towards size of droplet. The orthogonal design matrix for nine different MEs with three diameter readings taken per ME are given in Table above. The three-response data from each ME was used to obtain the signal/to noise ratio (S/N ratio) using equation provided in the methodology section. This transformation consolidates the three-response data into a single number which is the S/N ratio which takes variability and mean into account. The following step is to identify the effect the of each parameter towards particle size by generating table based on the Sound/Noise ratio calculated above. This combinational effect each level of a parameter has towards droplet size can be observed in table 4.3 below and in the figures 4.7, 4.8, 4.9 and 4.10 below. After this the data is further analyzed to identify the significance of each factor towards droplet size. This analysis was done by applying ANOVA analysis method. The percentage of which each factor influences the droplet size is shown in the table 4.3 below.

Table 4.3: Combinational Effect of Parameters on Droplet Size

Parameter	Name	Level 1	Level 2	Level 3
P1	Ratio of Tween-80:Span-20	28.39	32.04	38.71
P2	Concentration of Surfactant in IPM (%)	34.67	33.64	30.82
P3	IL Contend (wt%)	31.93	32.77	34.44
P4	Temperature (°C)	31.44	33.55	34.14

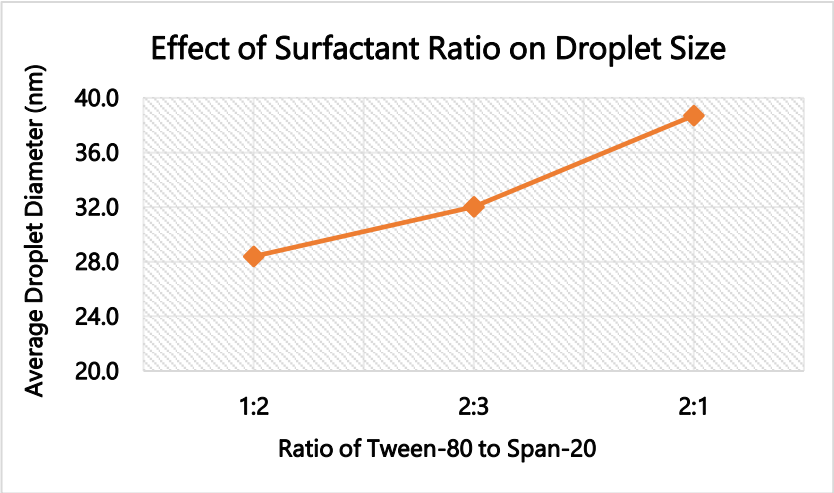

Figure 4.7: Effect of Surfactant Ratio on Droplet Size

The figure 4.7 above shows the effect that the surfactant ratio has on droplet size. It is observed that the droplet size increases as the ratio of Tween-80 to Span-20 increases. This means that the average droplet size increases as the concentration of Tween-80 increases and the concentration of Span-20 decreases in the surfactant mixture. In a solution with low concentration of Tween-80 the interactions between Tween chains are negligible. However, at higher concentration of Tween-80 the polyoxyethylene (POE) chains on the headgroup of the surfactant start to experience steric interactions and they impose a larger molecular area (Lu et al., 2000). Adding on to this justification, when a surfactant solution has a high concentration of Span-20, micelles will start to form just from this single surfactant. This will cause the average size of droplets to reduce compared to the average droplet size in a mixture of surfactants. A similar relationship between surfactant ratio and droplet size has also been seen in other studies (Moniruzzaman, 2010c).

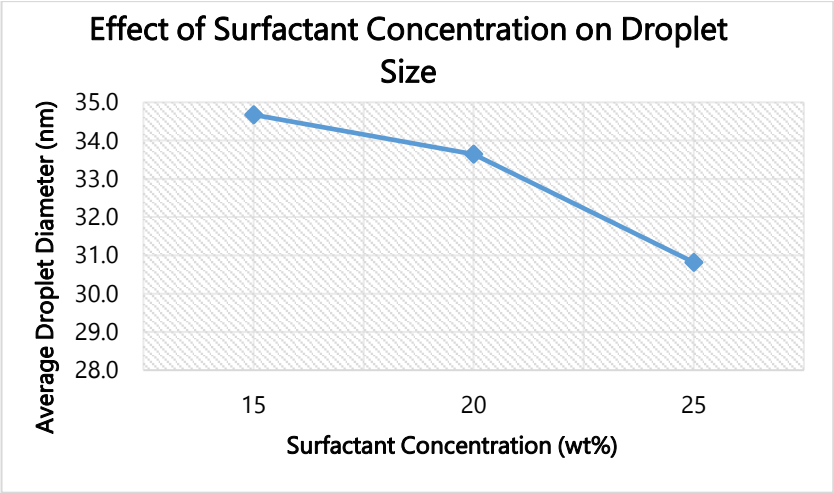

Figure 4.8: Effect of Surfactant Concentration on Droplet Size

The second parameter investigated was surfactant concentration. Surfactant concentration is defined as the concentration in weight percent of surfactant in the ME. It is observed that an increase in surfactant concentration leads to a decrease in droplet size. Surfactants which contain both hydrophilic groups and hydrophobic groups could make a stable barrier at the interface, acting as a stabilizer. The barrier enables the surfactants to prevent crystal growth by providing steric repulsion between particles. Therefore, the enough amount of surfactant is needed for coverage on the particles. Similar results was also seen in other studies where an increase in surfactant concentration leads to a decrease in droplet size (Seo et al., 2015; Chanana, 1995).

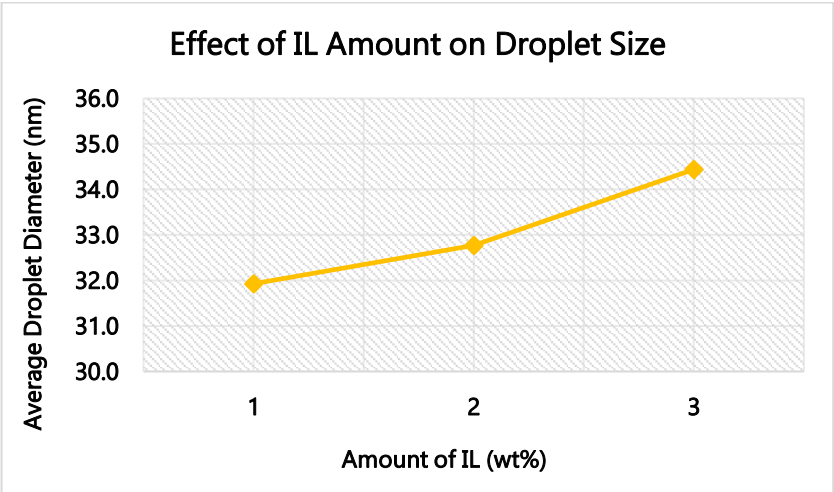

Figure 4.9: Effect of IL Amount on Droplet Size

The third parameter that was investigated was the effect of ionic liquid content towards droplet size. It was observed that the increase in ionic liquid content in a microemulsion leads to an increase in droplet size. This relationship is commonly seen in IL/O microemulsions (Moniruzzaman, 2010b; Gao et al., 2006; Eastoe et al., 2005). The data obtained shows that the IL is in the droplet core. Hence, an extra amount of IL leads to the surfactants aggregates to enlarge. This same phenomenon is typically observed in conventional ME (Pileni, 1989). This system contains droplets 25–130 nm in diameter, that are like the droplets formed in typical reverse micelles with water core stabilized by common surfactants (Luisi, 1985). This is an important finding due to the recorded sizes are significantly low compared to [C_2mim][BF]/TX-100/oil microemulsions (another type of IL/O ME), where size of droplets are in the range of 0.1µm (Eastoe et al., 2005) . These results prove the efficiency of Tween-80 and Span-20 surfactant blend in forming IL supported MEs.

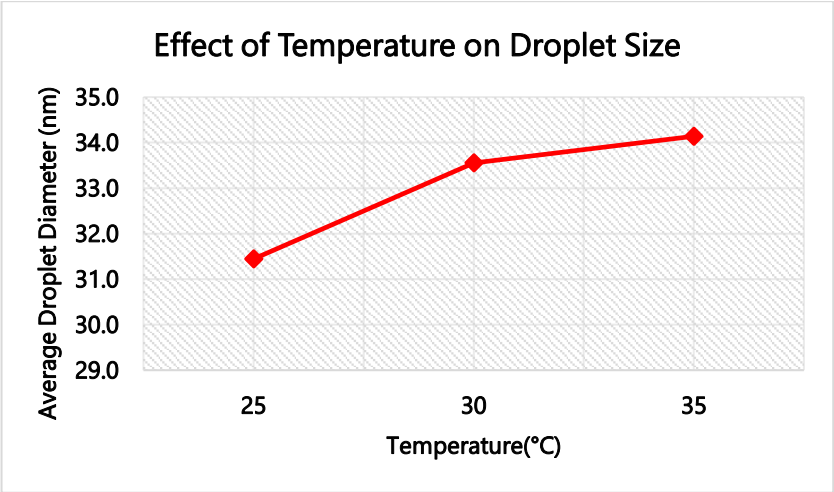

Figure 4.10: Effect of Temperature on Droplet Size

The final parameter that was investigated was the effect of temperature on the size of droplets. The data obtained states that the droplet size increases as the temperature increases. The size of the droplet increases linearly with temperature due to regular swelling of the micelles. IL/O MEs have higher temperature stability compared to other microemulsions as the ME is stabilized by temperature insensitive electrostatic interactions between ionic liquids and the polar head groups of Tween-80 and Span-20 (Huddleston, 2001). Other than the surfactant, the choice of cation also has significant impact on the extent to which temperature can influence the size of ionic liquid droplets (Seo et al., 2015). A microemulsion composed of imidazolium ionic

liquids with longer alkyl tails are less sensitive to changes in temperature, owning to the rigidity imposed by the alkyl chains on the interfacial surfactant layer (Seo et al., 2015).

Based on the data obtained above the parameters that will provide the lowest droplet size can be determined. The lower droplet size is preferred because low droplet size has a higher amount of skin permeation for transdermal drug delivery (Pattarino et al., 1994). The best conditions of parameters to get the lowest droplet size is as shown in the table 4.4 below.

Table 4.4: Optimized Level for Lowest Droplet Size

Parameter	Name of Parameter	Optimized Level for Lowest Droplet Size
P1	Ratio of Tween-80:Span-20	1:2
P2	Concentration of Surfactant in IPM (%)	25
P3	IL Contend (wt%)	1
P4	Temperature (°C)	25°C

After identifying the optimized levels to get the lowest droplet size, the next phase is to identify the parameter that has the most impact towards droplet size. This method is useful as it is important to identify which factors will give the most significant impact towards the droplet size. This is done by using ANOVA analysis and using equation that is given in Chapter 3 Methodology. The results of this analysis are stated in table 4.5 below.

Table 4.5: Significance of each Parameter towards Droplet Size

Column	Factors	DOF	Sum of Squares	Variance	Percent
P1	Ratio of Tween-80:Span-20	2	164.377	82.189	49.787
P2	Concentration of Surfactant in IPM (%)	2	53.934	26.967	16.336
P3	IL Contend (wt%)	2	99.798	49.899	30.227
P4	Temperature (°C)	2	12.052	6.026	3.650
	Total	**8**	**330.162**		**100.000**

The table 4.5 above shows the percentage of impact that each factor has towards droplet size and it can be observed that the surfactant ratio has the highest impact towards droplet size followed by IL content and surfactant concentration which has the second and third highest impact. The parameter with the least impact is temperature. The results above are useful as it can provide a method to control the droplet size to fit specific application and for this purpose a pareto analysis is done. The pareto analysis will identify which parameters need to be controlled to have a 80% impact towards the final output (droplet size). The pareto analysis can be observed from figure 4.11 below.

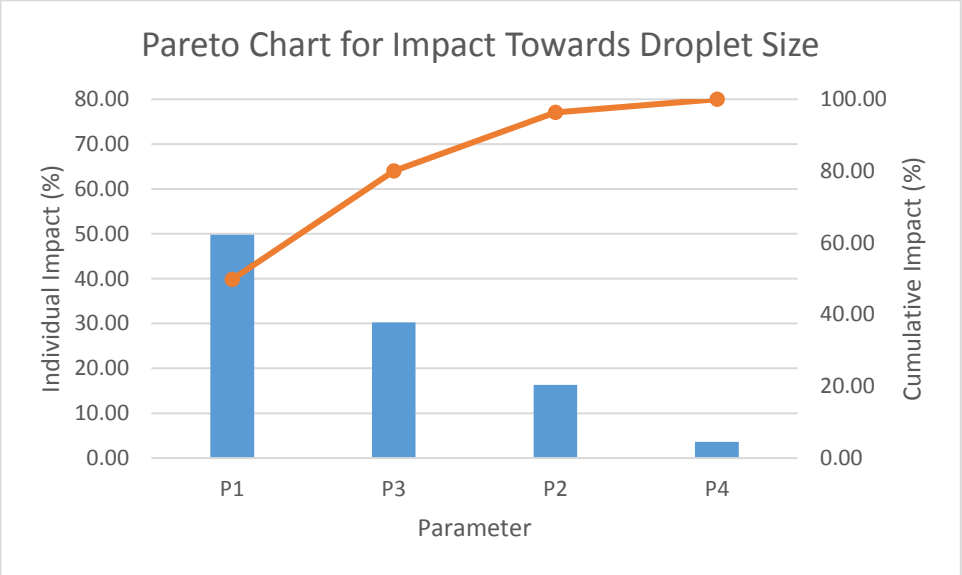

Figure 4.11: Pareto Chart for Impact of Parameters towards Droplet Size

Figure 4.11 above shows the pareto chart acquired from the pareto analysis. Based on pareto analysis theory, by selecting and controlling a small number of tasks a significant overall effect can be produced. The basic theory is by using only 20% of effort, an impact of 80% can be achieved. For the results shown in figure 4.11 it can be seen the cumulative impact of P1 and P3 is 80.01% which means that by focusing on these two parameters, a maximum of 80% impact can be achieved towards the droplet size. P1 is the ratio of surfactant and P3 is the IL content.

4.3 Solubility of ACV in IL/O microemulsion

In order to investigate the potential of IL/O MEs as an agent to dissolve drugs with poor solubility, the solubility of ACV which has poor solubility is studied. Although drugs such as ACV contain many polar groups in their molecular structure, their solubility in aqueous solutions have always been poor. Furthermore, the many polar groups on the molecule indicates low solubility in a-polar liquids, including lipid rich and surfactant rich mixtures. ACV was dissolved in 9 different MEs based on Taguchi design, bulk of ionic liquid [C_2mim][CH_3COO], water and IPM. Table 4.6 below shows the data on solubility of ACV in various MEs, [C_2mim][CH_3COO], water and IPM . Each microemulsion was prepared using different levels of each parameter based on Taguchi orthogonal array. Each experiment was repeated once again to ensure reproducible results. Table 4.6 and figure 4.12 below shows the standard curve obtained from the UV spectrophotometer at 252nm for identification of ACV solubility.

Table 4.6: Data on the Standards used in UV Spectrophotometer

Standard	Absorbance (A)	Solubility (mg/mL)
Standard 1	0.0305	0
Standard 2	0.3398	0.305
Standard 3	0.8438	0.802
Standard 4	10.1715	10

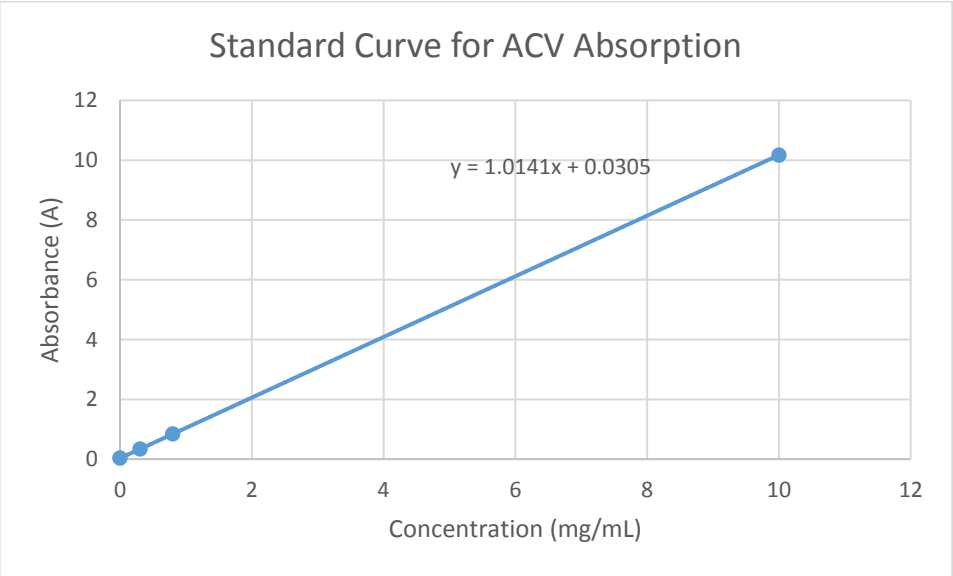

Figure 4.12: Standard Curve for ACV Adsorption

Table 4.7: Solubility of ACV in respective Microemulsions

Systems	Absorbance (A)	Solubility (mg/mL)
ME1	2.282	2.205
ME2	3.042	2.995
ME3	3.996	4.205
ME4	3.205	3.145
ME5	3.519	3.445
ME6	3.144	3.09
ME7	4.219	4.17
ME8	3.245	3.155
ME9	3.499	3.43
Water	0.558	0.52
IPM	0.114	0.082

The table 4.7 above shows the absorbance value obtained from the UV Spectrophotometer. The equipment was calibrated using the standards as stated in table 4.6 and the standard curve was plotted as shown in figure 4.12. The value of solubility stated in table 4.7 is taken from the average of two runs (S_1 and S_2). The solubility of ACV in water and IPM was also tested and it shows that ACV has higher solubility in the MEs compared to water and IPM.

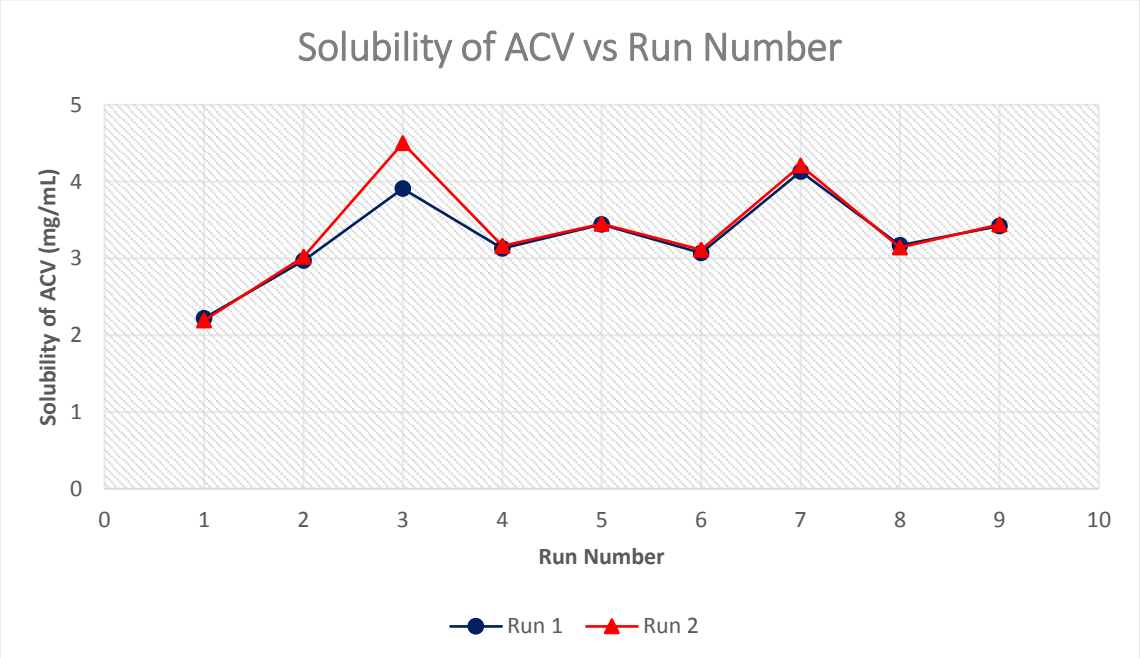

Figure 4.13: Solubility of ACV in all MEs by runs

Table 4.8: Data of Solubility and Signal to Noise Ratio for all nine MEs

ME	Process parameter				Results			
	P_1	P_2 (wt%)	P_3 (wt%)	P_4 (°C)	S_1 (nm)	S_2 (nm)	S_{avg} (nm)	Signal / Noise
1	1:2	15	1	25	2.22	2.190	2.205	6.868
2	1:2	20	2	30	2.97	3.020	2.995	9.527
3	1:2	25	3	35	3.91	4.500	4.205	12.411
4	2:3	15	2	35	3.13	3.160	3.145	9.952
5	2:3	20	3	25	3.44	3.450	3.445	10.744
6	2:3	25	1	30	3.07	3.110	3.09	9.799
7	2:1	15	3	30	4.13	4.210	4.17	12.402
8	2:1	20	1	35	3.17	3.140	3.155	9.980
9	2:1	25	2	25	3.42	3.440	3.43	10.706

The three-response data from each ME was used to obtain the signal/to noise ratio (S/N ratio) using equation provided in the methodology section. This transformation consolidates the three-response data into a single number which is the S/N ratio which takes variability and mean into account as shown in table 4.8. The following step is to identify the effect the of each parameter towards ACV solubility by generating table 4.8 based on the Sound/Noise ratio calculated above. This combinational effect each level of a parameter has towards droplet size can be observed in table 4.9 below and in the figures 4.14, 4.15, 4.16 and 4.17. After this the data is further analyzed to identify the significance of each factor towards droplet size. This analysis was done by applying ANOVA analysis method. The percentage of which each factor influences the droplet size is shown in the table below.

Table 4.9: Combinational Effect of Each Parameter Towards ACV Solubility

Parameter	Name	Level 1	Level 2	Level 3
P1	Ratio of Tween-80:Span-20	9.60	10.16	11.03
P2	Concentration of Surfactant in IPM (%)	9.74	10.08	10.97
P3	IL Contend (wt%)	8.88	10.66	11.85
P4	Temperature (°C)	9.44	10.58	10.78

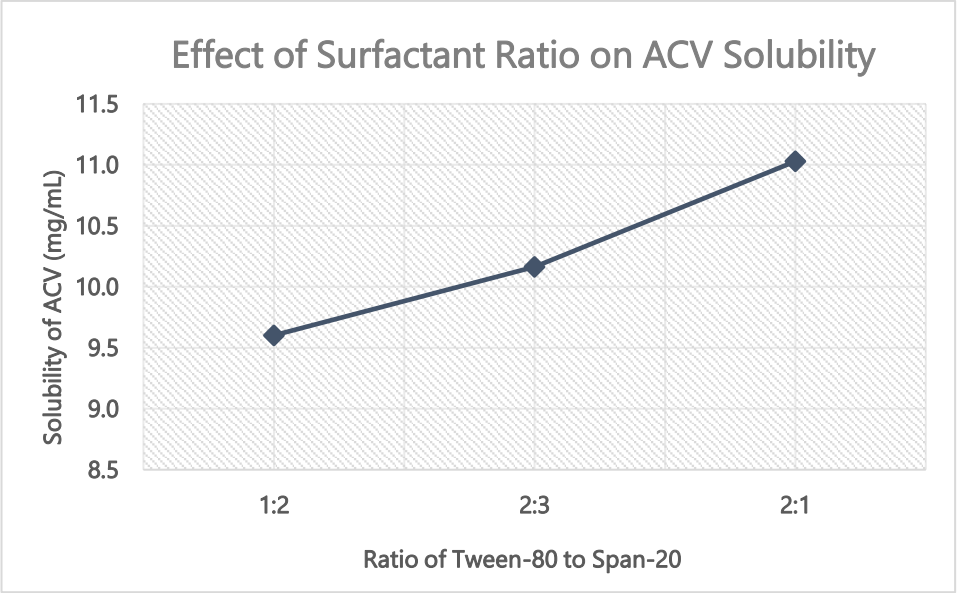

Figure 4.14: Effect of Surfactant Ratio on ACV Solubility

To investigate and assess IL/O MEs as a delivery system for sparingly soluble drugs, their solubility was determined and the effects surfactant ratio, surfactant concentration, ionic liquid content and temperature towards solubility was investigated. The first parameter that was investigated was surfactant ratio where three different ratios of Tween-80 and Span-20 was investigated since drug solubility in MEs is dependent on the content of individual surfactant (Kim et al., 2005). The solubility of ACV in bulk ionic liquid [C_2mim][CH_3COO] was found to be very low (about 1.612 mg/mL). In the presence of IL to form IL/o MEs, a remarkably high amount of ACV can be incorporated into MEs as shown in Table above via the solubilizing in the ionic liquid dispersed phase. The solubility of ACV was found to

vary on the Tween-80 to Span-20 weight ratios. Higher Tween-80 to Span-20 ratios lead to better solubilizing of ACV in the formulations. Particularly, when the weight ratios were below 2:3, the solubility of ACV was reduced significantly. This large increase in ACV solubility using ratio 2:1 is thought to be caused by the formation of stable ME droplets with large interface compared to that of others MEs (Narang et al., 2007).

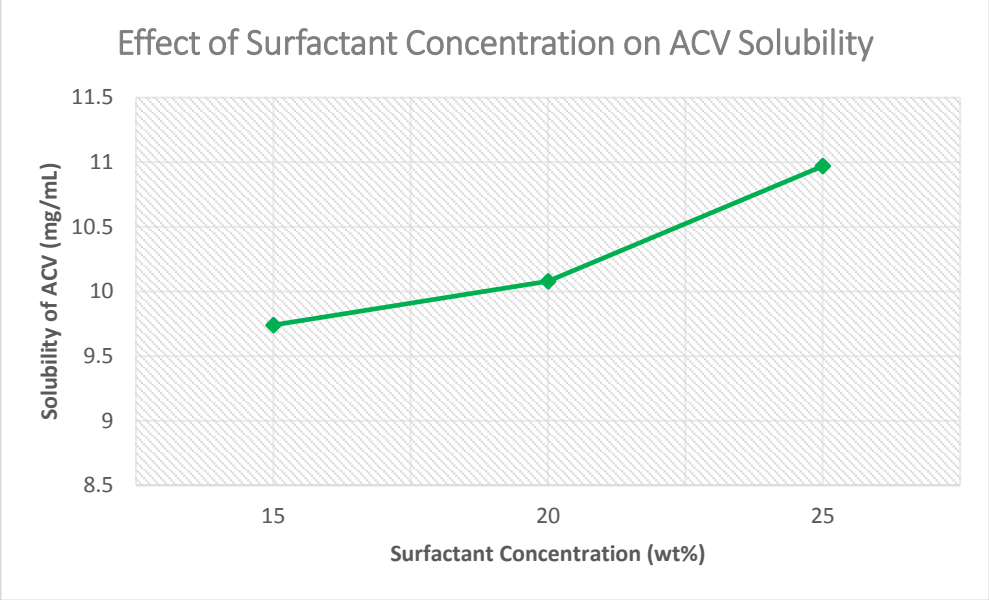

Figure 4.15: Effect of Surfactant Concentration on ACV Solubility

The second parameter that was investigated was surfactant concentration and it was found that an increase of surfactant concentration leads to an increase in ACV solubility. The higher concentration of surfactant means that there are more droplets formed due to reduced interactions between Tween-80/Span-20 particles with the particles in the dispersed phase (Moniruzzaman, 2010b). The reduction in interactions between surfactant in dispersed phase leads to increased interactions with solute particles and hence increasing the solubilizing ability of the ME. Similar results was also seen in other studies where an increase in surfactant concentration leads to an increase in drug solubility (Narang, 2005).

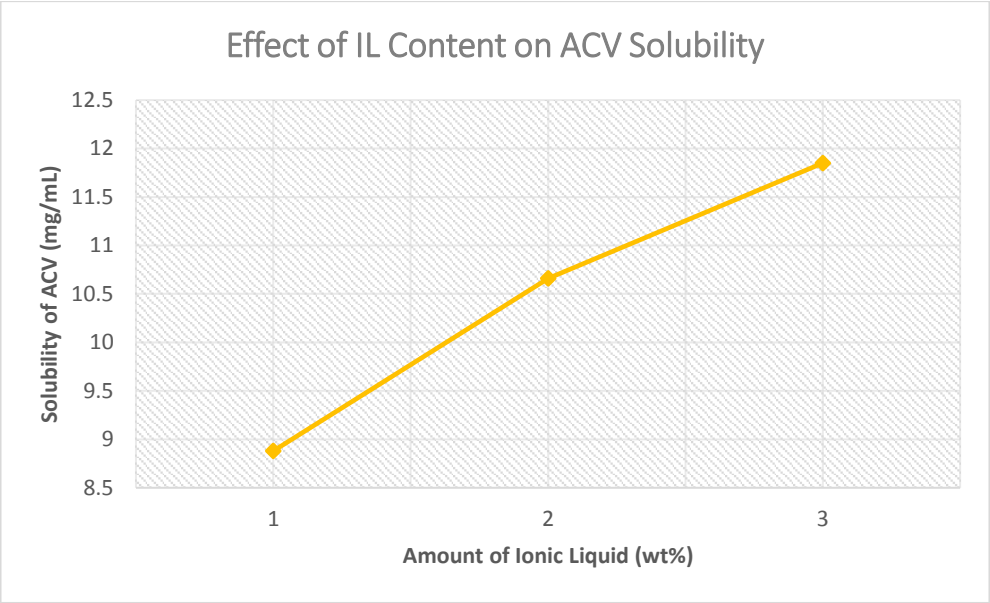

Figure 4.16: Effect of IL Content on ACV Solubility

The third parameter that was investigated was the content of ionic liquid. Based on the data obtained it was observed that an increase in ionic liquid content leads to an increase in solubility of drug. These results support that ionic liquids plays the main role in the solubilizing of drugs in ionic liquid-based MEs. Since the solubility of all drugs in IPM is very low, it can be assumed that the drugs molecules exist in the hydrophilic IL core, which is stabilized by the surfactants mixtures creating an interfacial film between IPM and ionic liquid.

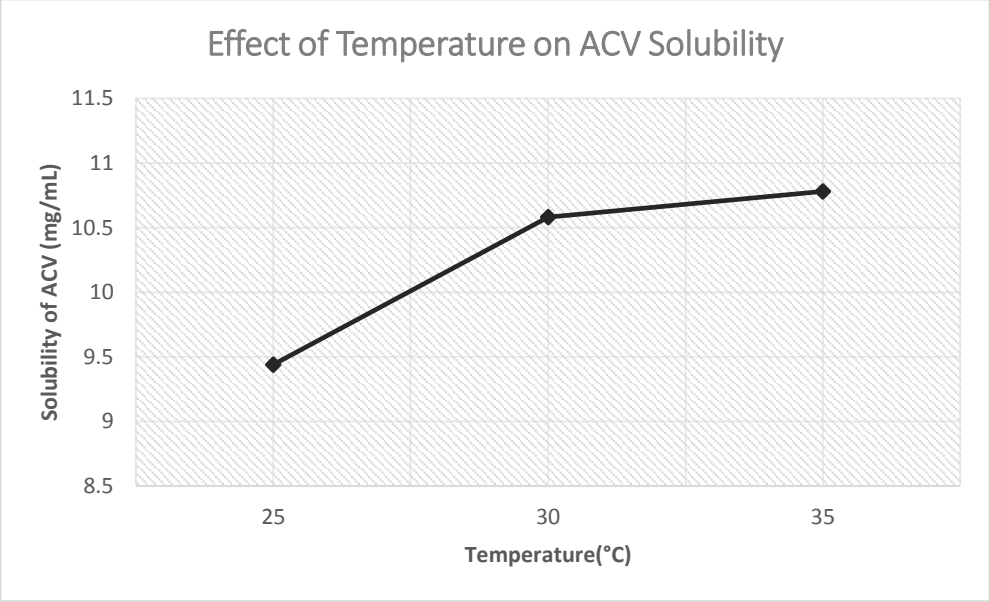

Figure 4.17: Effect of Temperature on ACV Solubility

The final parameter investigated that was investigated was temperature. It was observed that the increase of temperature leads to an increase in solubility. This can be further discussed by applying the basic theory of solubility. The increase in temperature leads to an increase in kinetic energy of particles in the ME and these particles collide more frequently with each other. Since it has been established that the drugs molecules exist in the hydrophilic IL core, the increase collision between the ionic liquid core and drug solute leads to an increase in drug solubility.

Based on the data obtained above the parameters that will provide the highest ACV solubility can be determined. The highest ACV solubility is preferred so more amount of drug can be delivered with a low amount of ionic liquid. The best conditions of parameters to get the highest ACV solubility is as shown in the table 4.10 below.

Table 4.10: Optimized Level for Highest ACV Solubility

Parameter	**Name of Parameter**	**Optimized Level for Highest ACV Solubility**
P1	Ratio of Tween-80:Span-20	2:1
P2	Concentration of Surfactant in IPM (%)	25
P3	IL Contend (wt%)	3
P4	Temperature (°C)	35°C

After identifying the optimized parameters that gives the highest ACV Solubility, the next phase is to identify the parameter that has the most impact towards ACV solubility. This method is useful as it is important to identify which factors will give the most significant impact towards the solubility. This is done by using ANOVA analysis and using equation that is given in Chapter 3 Methodology. The results of this analysis are stated in table 4.11 below.

Table 4.11: Significance of each Parameter towards ACV Solubility

Column	Factors	DOF	Sum of Squares	Variance	Percent
P1	Ratio of Tween-80:Span-20	2	9.100	4.550	34.896
P2	Concentration of Surfactant in IPM (%)	2	2.423	1.212	9.293
P3	IL Contend (wt%)	2	13.419	6.710	51.459
P4	Temperature (°C)	2	1.135	0.568	4.352
	Total	**8**	**26.078**		**100.000**

The table 4.11 above shows the percentage of impact that each factor has towards ACV solubility and it can be observed that the IL content has the highest impact towards droplet size followed by surfactant ratio and surfactant concentration which has the second and third highest impact respectively. The parameter with the least impact is temperature. The results above are useful as it can provide a method to control the solubility to fit specific application and for this purpose a pareto analysis is done. The pareto analysis will identify which parameters need to be controlled to have 80% impact towards the final output (ACV solubility). The pareto analysis can be observed from figure 4.18 below.

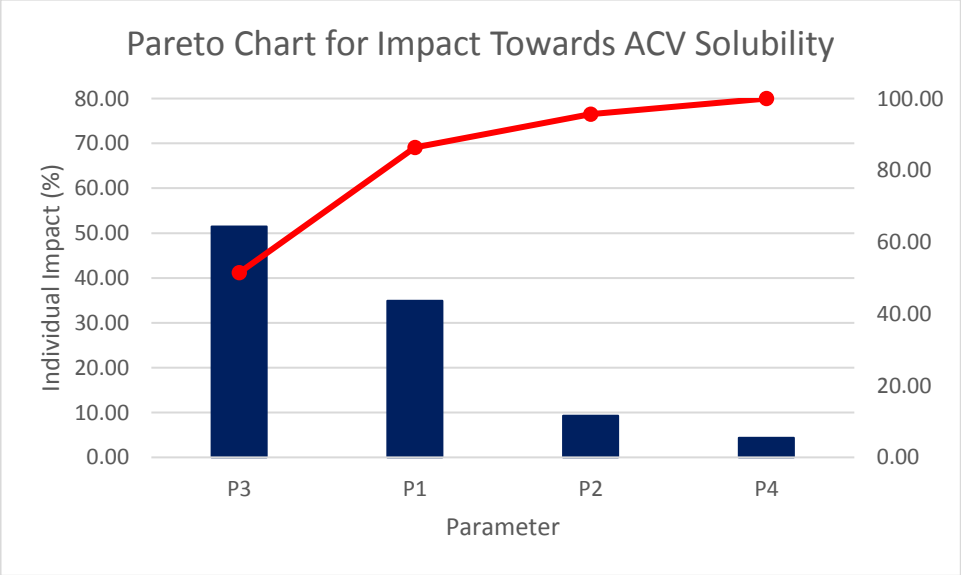

Figure 4.18: Pareto Chart for Impact of Parameters towards ACV Solubility

Figure 4.18 above shows the pareto chart acquired from the pareto analysis. Based on pareto analysis theory, by selecting and controlling a small number of tasks a significant overall effect can be produced. The basic theory is by using only 20% of effort, an impact of 80% can be achieved. For the results shown in figure 4.18 it can be seen the cumulative impact of P3 and P1 is 86.36% which means that by focusing on these two parameters, a maximum of 80% impact can be achieved towards the droplet size. P1 is the ratio of surfactant and P3 is the IL content.

4.4 Final Optimized Parameters

This study aims to optimize the parameters in such away where the microemulsion formed will have small droplet size and high ACV solubility. Smaller droplet size is preferred as it has higher ability to permeate through the skin. For solubility, the higher is preferred as more drug can be transferred in a small amount of ionic liquid. The final optimized parameters lead to a high drug solubility and low droplet size. The final optimized parameters are given as the table 4.12 below.

Table 4.12: Final Optimized Levels

Parameter	Name of Parameter	Final Optimized Parameters
P1	Ratio of Tween-80:Span-20	2:3
P2	Concentration of Surfactant in IPM (%)	25
P3	IL Contend (wt%)	2
P4	Temperature (°C)	30°C

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Transdermal drug delivery is an important method of drug delivery and the main problem it faces is the low solubility of many drugs in water and most organic solvents. It has been proven that by usage of ionic liquid in oil microemulsion these drugs can be solubilized and used for transdermal drug delivery. Usage of microemulsions as the carrier system for the drug into the body is a promising idea. The problem with the previous studies is that the components used are not pharmaceutically accepted. This current study uses pharmaceutically accepted components that have low toxicity to develop microemulsions. The effect of four parameters namely surfactant ratio, surfactant concentration, IL content and temperature towards droplet size and drug solubility. It was concluded that as surfactant ratio increases (increasing Tween-80), both the droplet size and drug solubility increases. It was also observed as the surfactant concentration increases there is an increase in drug solubility but there is a decrease in particle size. Third observation made was that as the IL content increases there is an increase in both particle size and drug solubility. The final observation is that as the temperature increases the drug solubility and droplet size increase. The parameter that influences particle size the most is surfactant ratio whereas the parameter that influences the drug solubility the most is IL content. The parameters were further optimized to identify the conditions that will give the highest drug solubility and lowest droplet size. It was concluded that the optimized conditions can be achieved by using surfactant ratio as 2:3, surfactant concentration as 25wt%, ionic liquid content at 2wt% and temperature at 30°C.

5.2 Recommendations

There are a few expected limitations that will be encountered in this project. Firstly, the time given for the completion of this project is 8 months and this time includes the writing of reports and presentations. 8 months in final year is a very short time and minus the time will be taken to write reports this will leave little time for experimentation and more intensive research on this topic. Secondly the scope of this project is limited to microemulsion formulation and characterization and it does not cover the skin permeation test due to the time constraint. In future studies relating to this title, skin permeation studies should be done for the microemulsion formed using the optimized parameters. This will give experimental evidence that the microemulsion formed can indeed be a useful innovation in transdermal drug delivery. Finally, in this study the cytotoxicity studies of the microemulsion with the optimized parameters was not conducted. There is data from other studies stating that the material used such as ionic liquid 1-ethyl-3methylimidazolium acetate, the surfactants and IPM have low toxicity. However, to further strengthen the data obtained in this study the final microemulsion formed should be tested for its cytotoxicity.

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APPENDICES

APPENDIX A: Gantt Chart for Final Year Project 1 (14 weeks)

Week / Activities	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.0 Selection & Finalization of Project Title	X	X												
2.0 Meeting with Supervisor		X	X	X	X	X	X	X	X	X	X	X	X	X
2.0 Preliminary Research Work														
2.1 Desk Study			X	X										
2.2 Setting Objectives					X									
2.3 Literature Review					X	X								
3.0 Submission of Extended Proposal							X							
4.0 Research Proposal Defense									X					
5.0 Project Work Continue														
5.1 Selection of ILs										X	X	X		
5.2 Solubility Study													X	X
5.3 Selection of Surfactants												X	X	X
6.0 Submission of Interim Draft Report													X	
7.0 Submission of Interim Report														X

APPENDIX B: Gantt Chart for Final Year Project 2 (14 weeks)

Week / Activities	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.0 Meeting with Supervisor	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2.0 Project Work Continue														
2.1 Identify Taguchi Orthogonal Design Parameters	X	X												
2.2 Finalize the Taguchi Orthogonal Design Parameters			X	X										
2.3 Microemulsion Preparation				X	X									
2.4 Characterization of Microemulsion					X	X								
2.5 Final Data Analysis						X	X							
3.0 Progress Report				X	X									
4.0 Pre – SEDEX														
4.1 Preparation for Pre – Sedex								X	X					
4.2 Qualification for Pre – Sedex									X	X				
5.0 Technical Paper										X	X			
6.0 Dissertation											X	X		
7.0 Viva												X	X	
8.0 Submission of Dissertation														X

APPENDIX C: Key Milestones of Project