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Preparation and Characterization Of Oil-in-water Nano-emulsions Of Trifluoperazine For Parenteral Drug Delivery

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PREPARATION AND CHARACTERIZATION OF OIL-IN-WATER NANO-EMULSIONS OF TRIFLUOPERAZINE FOR PARENTERAL DRUG DELIVERY

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By

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ABSTRACT

PREPARATION AND CHARACTERIZATION OF OIL-IN-WATER NANO-EMULSIONS OF TRIFLUOPERAZINE FOR PARENTERAL DRUG DELIVERY

By

Toluwalope Onadeko

May 2009

Thesis Supervised by Moji Christianah Adeyeye, PhD

Objectives: 1.) To develop and characterize an optimal formulation of oil-in-water nano-emulsions of trifluoperazine for parenteral delivery. 2.) To perform short term stability testing of the optimal formulation and monitor the potency using high performance chromatography (HPLC).

Materials and Methods: Emulsions containing soybean oil, water, trifluoperazine hcl as an amphiphilic drug, phospholipon 90 and Tween 80 as surfactant blend were prepared using the Thin-layer hydration method. Z-average, polydispersity index, zeta potential of emulsions were determined. A fully randomized 2X2X2X2X2 statistical design was developed using JMP software. Optimal formulation was selected based on desirable properties of low z-average and polydispersity index, and high zeta potential. Stability of optimal formulation was determined using HPLC analysis and based on ICH specifications.

Results: Z-average of optimal formulation was 72.9nm with zeta potential value of 25.59 mV and polydispersity index 0.2. After storage for 3 months, z-average values were below 200nm indicating optimal formulation was not physically degraded. Drug content analysis showed chemical degradation due to reduction of potency.

Conclusions: Trifluoperazine nano-emulsions formulations had acceptable values of low z-average, low polydispersity index and high zeta potential and were physically stable but not chemically stable over 3 months.

DEDICATION

This work is dedicated to my family.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

AMVn	Automated Microviscometer
AP	Aqueous Phase
CRT	Cathode Ray Tube
CMC	Critical Micelle Concentration
EPC	Egg Phosphatidyl Choline
Exp.	Experiment
HEPC	Hydrogenated Egg Phosphatidyl Choline
PI	Polydispersity
P90	Phospholipon 90
HLB	Hydrophilic- Lipophilic Balance
HPLC	High Performance Liquid Chromatography
Log.	Logarithm
ODS	Octadecyl Silica
o/w	Oil-in-water
NMR	Nuclear Magnetic Resonance
PVC	Polyvinyl Chloride
SEM	Scanning Electron Microscopy
T80	Tween 80
TEA	Triethylethanolamine
TEM	Transmission Electron Microscopy
TP	Trifluoperazine
TPN	Total Parenteral Nutrition

USP	United States Pharmacopeia
Z.P	Zeta Potential

1.0 Introduction

1.1 Statement of the Problem

Development of intravenous lipid emulsions began in the 1920's; however, none of the early fat emulsions could be used safely in man. Since then, a very large number of emulsions with various lipids and emulsifiers have been investigated (1). In the early 1960s, Arvid Wretlind and co-workers developed the first safe parenteral lipid based emulsion for clinical nutrition, called Intralipid® (2). This product provides concentrated energy and essential fatty acids to patients who are unable to eat. By focusing on the formulation technology and the selection and purification of the lipid raw materials, this group of researchers overcame several adverse clinical reactions that had caused extensive problems for earlier investigators, including withdrawal of products from the market. The basic objective is to mimic as well as possible the input of chylomicrons into the blood. The clinical use of parenteral fat emulsions is now globally accepted as a part of nutrition therapy. Intralipid became the starting point for using lipid emulsions as a delivery matrix for lipid-soluble drugs (3, 4).

Studies have shown that nano-emulsions can prolong drug circulation in the plasma by preventing drug uptake by the liver in to the reticuloendothelial system (5). Their potential applications range from antifungal delivery agents to immunodulators (6,7).

These delivery systems may also be used for localized therapy, such as in the treatment of lymph node metastases, or injection in to other cavities and joints (8).

There are several advantages to the use of parenteral lipid emulsions as drug carriers. These include stabilization of drugs that are susceptible to hydrolysis, enhancement of solubility for water insoluble drugs and reduction of sorption of aqueous drug on plastic material of infusion set. The use of oil-in-water emulsion can improve aqueous solubility by incorporating the insoluble drug into the interior oil phase. Protein binding and hydrolytic degradation of certain drugs such as barbiturates do not occur as long as the drug remains in the oil phase thus further improving the therapeutic efficacy for emulsion formulations compared with aqueous solutions. Direct contact of such a drug in a parenteral emulsion with the body fluids and tissues is reduced and the drug may be released slowly over a prolonged period of time; this may minimize side effects (9, 10, 11).

There is a potential for sustained release of drugs from parenteral emulsion (12, 13). Delayed absorption of the total dose from an emulsion can be achieved for drugs with large partition coefficient. The fraction of drug available for absorption also depends on the phase volume ratio between the lipid and aqueous phases in the delivery system. For example, if the volume of the aqueous phase is much larger than that of the oil phase, a large partition coefficient (w/o) will result in a small fraction of the insoluble drug being available for absorption and hence a sustained release effect (14).

Trifluoperazine was chosen as a model drug that could be delivered in a lipid emulsion because of its favorable properties. It is an amphiphilic drug, therefore, a good

candidate for an emulsion because it will tend to localize at the oil/water interface and intercalate in the phospholipids monolayers due to its surface active properties (15). Its high log P value indicates that more of the drug will be distributed in the oil phase. Formulation of trifluoperazine emulsion may enhance the solubility of the drug compared with an aqueous formulation of the drug.

Commercially available aqueous parenteral preparation has been reported to be unstable and subject to discoloration with exposure to light and sorption of trifluoperazine hydrochloride to infusion sets has been reported in literature. Trifluoperazine hydrochloride 10mg/ml in sodium chloride 0.9% exhibited a cumulative 45% loss during a seven hour simulated infusion through an infusion set consisting of a cellulose propionate chamber and 170 cm of PVC tubing due to sorption. The extent of sorption was found to be independent of concentration. (16). An oil-in-water emulsion formulation of trifluoperazine may improve the delivery and chemical stability of the drug by reducing the likelihood of sorption of the drug on to infusion sets since most of the drug will be incorporated in to the oil phase of the emulsion compared with an aqueous formulation. Higher drug load may also be possible compared to the aqueous solution. This is due to its high log P as stated earlier. Therefore, the aims of the project were;

1. To prepare oil-in-water emulsions for parenteral delivery using excipients which are generally regarded as safe (GRAS).
2. To develop an optimal formulation with acceptable particle size in the nano range and evaluate other properties such as polydispersity index, zeta potential and viscosity.

3. To perform short term stability testing of the optimal formulation and monitor the potency using high performance chromatography (HPLC).

1.2 Literature Review

1.2.1 Emulsion Technology

An emulsion is a dispersed system containing at least two immiscible liquid phases. In order to ensure stability, an emulsion must contain a suitable emulsifying agent aside from the dispersed phase and dispersion medium (17).

Pharmaceutical emulsions usually consist of a mixture of an aqueous phase with various oils and or waxes. If the oil droplets are dispersed throughout the aqueous phase the emulsion is termed oil-in water (o/w). A system in which the water is dispersed throughout the oil is termed water-in-oil (w/o) emulsion. Multiple emulsions such as water-in-oil-in-water (w/o/w) can be formed. In this case, small water droplets are enclosed within larger oil droplets, which themselves are dispersed in water. The alternative o/w/o is also possible (18).

Emulsions with droplet size in the nanometric scale (typically in the range 20-200nm) are referred to in literature as miniemulsions, nano-emulsions, ultrafine emulsions, submicron emulsions, etc. The term nano-emulsions is preferred because it gives an idea of the nanoscale size range of the droplets; it is concise and it avoids misinterpretation with the term microemulsion which are thermodynamically stable systems (19, 20).

Parenteral emulsions are basically prepared in two ways. One is referred to as the extraneous addition of a lipophilic compound to a preformed sterile fat emulsion, usually with the assistance of an organic solvent such as ethanol or diethyl sulfoxide. This method is not recommended for several reasons such as formation of large poorly emulsified oil droplets, undissolved drug crystals and sterility problems. The second

method which is the preferred method is an ab initio emulsification of drug containing oil phase either aseptically or in conjunction with terminal heat sterilization. Thus, emulsions are custom designed to accommodate the unique requirements of a particular therapeutic agent (21).

1.2.2 Mechanisms of Emulsion Formation

In order to disperse one liquid in another in the form of an emulsion, an amount of work (W) in ergs/cm must be done upon the system. W is equal to the interfacial tension (γ) in ergs/cm^2 multiplied by the ΔS (cm^2) which is the increase in surface area of dispersed phase due to formation of emulsion droplets.

$$W = \gamma \Delta S \quad \text{……Equation 1}$$

To reduce the amount of energy required for emulsification and yet obtain small droplets, the interfacial tension between water and oil must be lowered to a marked degree. This can be achieved using appropriate emulsifying agent or and homogenization. To have thermodynamic stability, W must be very small. This implies that the surface area and interfacial tension must be small. However, to have a small specific surface area, the droplets must be large. This could lead to aggregation or coalescence and faster breakdown of emulsion. Therefore, there must be a balance of the thermodynamic and physical stability, i.e. keeping W as minimal as possible and keeping the z-average small enough (22).

Emulsion droplets are deformed and disrupted by viscous or inertial forces. Viscous forces generate tangential and normal stresses at the drop surface. Inertial forces

generate pressure differences. Laminar or turbulent flow and or cavitation forces may occur. In laminar flow, viscous forces are predominant. The flow can cause shear or elongation. In turbulent flow, inertial forces predominate; however, viscous forces may also be involved (23). During cavitation, small vapor bubbles are formed which subsequently collapse extremely fast, causing heavy shock waves in liquid. These may disrupt droplets. The liquid is intensely agitated and flow is turbulent. Hence, cavitation is comparable to disruption by turbulence.

The occurrence of any of these mechanisms depends on the type of apparatus, viscosity of liquid and constructional details. The scale of the apparatus may considerably affect the operation. A larger machine operated at appropriate speed gives more turbulence.

Emulsion droplets may also be disrupted due to interfacial instability caused by surface tension gradients. This is dependent on the surfactant and can occur in any equipment (24).

Emulsions can be prepared by high energy emulsification methods which includes high pressure homogenization and ultrasound generators or by low energy emulsification methods such as spontaneous emulsification or solvent diffusion method and phase inversion temperature (PIT) method (25).

1.2.2.1 Emulsification by Ultrasonication.

Emulsions can be produced using a sonicator, a laboratory equipment that generates ultrasonic waves. This type of sonicator has an ultrasonic transducer which consists of piezoelectric crystal contained within a protective metal casing. Application of

a high electrical wave to the transducer causes the piezoelectric crystal to rapidly oscillate and generate an ultrasonic wave. At the beginning of emulsification, waves are produced at the interface giving rise to fairly coarse drops. During this process of emulsification by ultrasound, cavitation or formation of cavities by liquid and their subsequent collapse occur accompanied by intense hydraulic shocks. (Figure 1.1) The exact mechanism of droplet break up is unknown. Postulations are that the droplet oscillates at a natural frequency until it bursts (26).

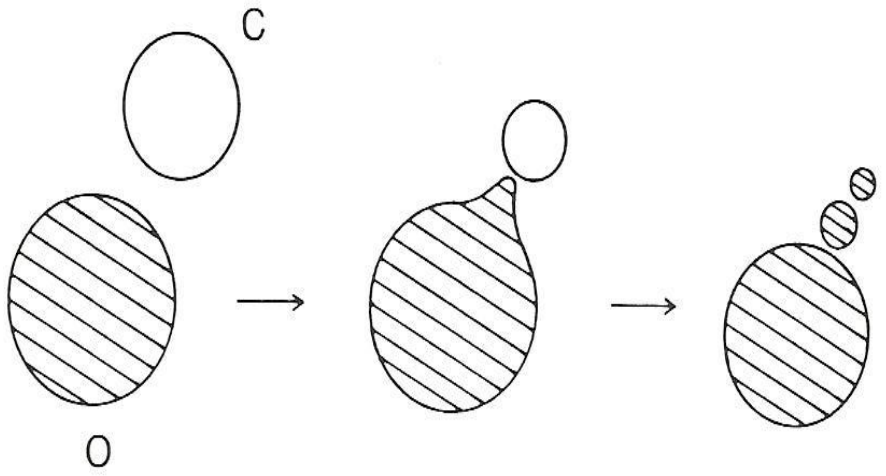

Figure 1. 1 Mechanism of Cavitation in Emulsification by Ultrasound (27)
(Cavity C collapses in the vicinity of droplet O)

Drug emulsions for parenteral delivery have been prepared by sonication. J. Medina et al. studied the development of a parenteral emulsion formulation for lorazepam using sonication (28). Jafari and coworkers evaluated the efficiency of sonication and microfluidization in the production of d-limonene oil-in-water nano-emulsions and observed that the size of the emulsions decreased with increasing sonication time and also produced emulsions in the size range of 150-700nm (29).

1.2.2.2 Emulsification using High Pressure Homogenizers.

Homogenizers may be used in one of two ways; 1.) By mixing ingredients in the emulsion and then passing those through the homogenizer to produce the final product and 2.) Produce a coarse emulsion first using a different method then passing it through a homogenizer. The purpose is to decrease droplet size and obtain a greater degree of uniformity and stability. The homogenizers operate at pressures of 1000 to 5000 psi to produce some of the finest dispersions obtainable in an emulsion (30).

1.2.2.3 Phase Inversion Method.

Phase inversion in emulsions can be one of two types: transitional inversion or catastrophic inversion (31). These methods make use of changing the spontaneous curvature of the surfactant. For non-ionic surfactants, phase inversion can be achieved by changing the temperature of the system, forcing a transition from an oil-in-water (O/W) emulsion at low temperatures to a water-in-oil (W/O) emulsion at higher temperatures (transitional phase inversion). During cooling, the system crosses a point of zero spontaneous curvature and minimal surface tension, promoting the formation of finely dispersed oil droplets. Catastrophic inversion is a transition in the spontaneous radius of curvature that can be obtained by changing the volume fraction of the phase. By successively adding water into oil, initially water droplets are formed in a continuous oil phase. Increasing the water volume fraction changes the spontaneous curvature of the surfactant from initially stabilizing a W/O emulsion to an O/W emulsion at the inversion locus. This process is well known for short-chain surfactants which form flexible

monolayers at the oil–water interface, resulting in a bicontinuous microemulsion at the inversion point (32).

1.2.3 Characterization of Emulsions

Characterization of nano-emulsions is of utmost importance in order to ensure the production of emulsions which fall within the desired droplet size range, viscosity and charge and are stable with time. Several techniques have been developed to characterize emulsions such as particle size analysis, polydispersity and zeta potential determination, differential scanning calorimetry, nuclear magnetic resonance (NMR), HPLC, viscosity and surface tension determination. Some of these methods will be highlighted below.

1.2.3.1 Particle size determination

Particle size of emulsions can be determined using several techniques. Some of the major techniques are hydrodynamic chromatography, photon correlation spectroscopy, spectroturbidimetry, field flow fractionation, sensing zone, electron microscopy and sedimentation (33).

1.2.3.1.1 Photon Correlation Spectroscopy

Photon correlation spectroscopy is also known as dynamic light scattering. The principle of operation is based on the intensity of the light scattered from dispersions of particles and macromolecules. This intensity fluctuates with time and are too rapid and shift too little to be evident to the human eye. The pace of the movement of particles is inversely proportional to the particle size and can be detected by analyzing the time

dependency of the light intensity fluctuations scattered from particles when they are illuminated with a laser beam. The time dependence of the light scattered from a very small region of solution over a time range from 10^{th} of a microsecond to milliseconds is measured. These fluctuations in intensity are related to the rate of diffusion of particles in and out of the region being studied (Brownian motion), and the data can be analyzed to directly give the diffusion coefficients of the particles doing the scattering. The data are processed to give the "size" of the particles (radius or diameter) which is based on the theoretical relationship between the Brownian motion and the size of the spherical particles (34). This is based on the Stokes-Einstein equation.

$$D_o = \frac{kT}{3\pi\eta d} \text{ ----------Equation 2}$$

where D_o is the diffusion coefficient, η is the viscosity of the suspending medium, and d is the particle diameter (35). The instrumentation for photon correlation spectroscopy shown in Figure 1.2 consists basically of a light source, which is either argon or helium-neon laser, a spectrometer consisting of an optical system for defining the scattering angle and limiting the number of coherence areas, a detector (usually a photomultiplier), a signal analyzer which is a digital autocorrelator and a computer for processing and displaying the data (36). Instruments such as Malvern Zetasizer Nanoseries® (Malvern Instruments, UK) and Nicomp® particle sizer (Particle Sizing Systems, CA) are available for measurement of particle size.

The diameter that is measured in dynamic light scattering is the hydrodynamic diameter, known as z-average. Z-average is the mean hydrodynamic diameter and is

calculated according to the international standard on dynamic light scattering ISO 13321. It is intensity weighted so it is sensitive to the presence of large particles (37).

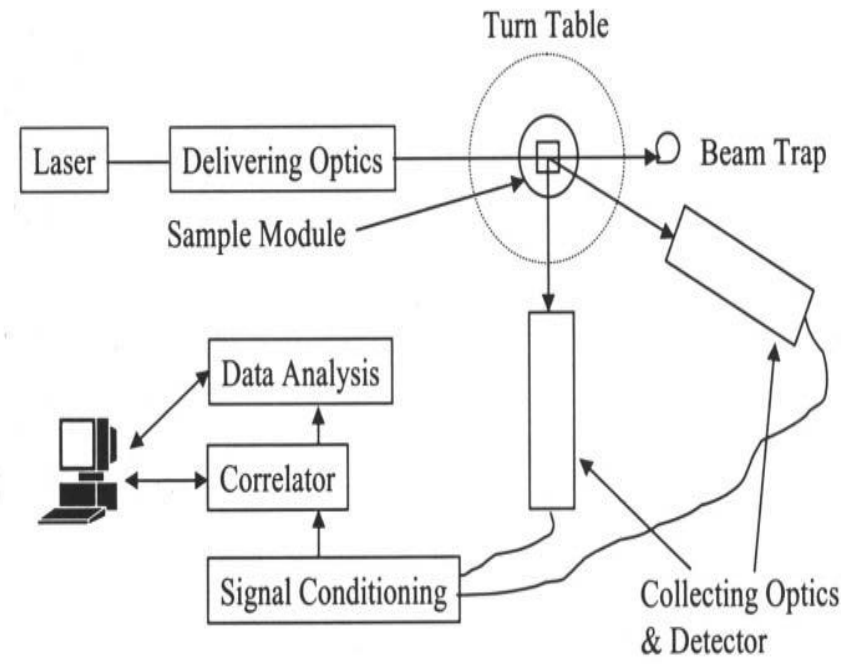

Figure 1. 2 Schematic diagram of a PCS instrument (38)

1.2.3.1.3 Electron Microscopy Techniques

Scanning electron microscopy and transmission electron microscopy are important techniques in emulsion characterization. Microscopic techniques allow determination of both particle size and distribution, and observation of particle shape. In

addition, other parameters, such as morphology or surface roughness can be observed (39).

1.2.3.1.3.1 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is one of the most widely used techniques used in characterization of nanomaterials and nanostructures. The resolution of the SEM approaches a few nanometers, and the instruments can operate at magnifications that are easily adjusted from - 10 to over 300,000. SEM produces topographical information and the chemical composition information near the surface. In a typical SEM, a source of electrons is focused into a beam, with a very fine spot size of 5 nm and having energy ranging from a few 100 eV to 50 Kev that is rastered over the surface of the specimen by deflection coils. As the electrons strike and penetrate the surface, a number of interactions occur that result in the emission of electrons and photons from the sample. SEM images of the emitted electrons are consequently produced on a cathode ray tube (CRT) (40).

1.2.3.1.3.2 Transmission Electron Microscopy (TEM)

In TEM, electrons are accelerated to 100 KeV or higher (up to 1 MeV), projected onto a thin specimen (less than 200nm) by means of the condenser lens system, and penetrate the sample thickness either undeflected or deflected. The greatest advantages that TEM offers are the high magnification ranging from 50 to10^6 and its ability to provide both image and diffraction information from a single sample.

1.2.3.2 Polydispersity Index

The second characterization of emulsion to be discussed is the polydispersity index. Photon correlation spectroscopy can also be used to determine the polydispersity index of an emulsion using the Malvern® Zeta sizer. This is necessary to characterize the particle size distribution of an emulsion. Polydispersity is the width of particle size. A narrow size distribution, corresponding to a polydispersity index between 0.1 and 0.2, is generally found with colloidal drug carriers. Larger polydispersity indices indicate a broad size distribution.

1.2.3.3 Zeta Potential Determination

Zeta potential is a measurement of surface potential. The magnitude of zeta potential gives an indication of potential stability of an emulsion (41). Zeta potential is an important parameter in determining the stability of an emulsion and other colloidal dispersions; zeta potential larger than about 25 mV is typically required to stabilize a colloidal system. Zeta potential is determined by a number of factors, such as the particle surface charge density, the concentration of counter ions in the solution, solvent polarity and temperature (42). Zeta potential can be determined using the Malvern Zeta sizer or the Nicomp particle sizer. Zeta potential is determined by electrophoretic light scattering (ELS). The Smoluchowski equation can be used to compute the zeta potential from electrokinetic mobility μ.

$$\mu = \zeta \varepsilon / \eta$$----------Equation 3

where ε is the permittivity and η the viscosity of the liquid used. (43)

1.2.3.4 Viscosity Determination

Viscosity is defined as a measurement of the applied stress per unit area required to maintain a certain flow rate or shear rate. In general, viscosity is the resistance to liquid flow, whereas fluidity is the reciprocal of viscosity or the coefficient of viscosity. Thicker liquids have higher the viscosities while thinner the liquids have lower viscosities or higher fluidity (44). Two types of viscosity may be specified: dynamic viscosity μ and kinematic viscosity ν. Dynamic viscosity is the absolute viscosity of the emulsion and the kinematic viscosity is obtained by dividing the dynamic viscosity by the density of the liquid. The kinematic viscosity is the viscosity due to the influence of gravity. They are related by the expression $\mu = \rho\nu$ where ρ is the density of the fluid (45). Viscosity can be determined by several methods such as rotational, falling or rolling ball, capillary tube or orifice and surface viscosity methods.

1.2.3.4.1 Rolling ball Viscometer

The rolling ball viscometer operates based on Stoke's law. When a body falls through a viscous medium it experiences a resistance or viscous drag which opposes the downward motion. Consequently if a body falls through a liquid under the influence of gravity, an initial acceleration period is followed by motion at a uniform terminal velocity when the gravitational force is balanced by the viscous drag (46).

The Stoke's equation can be used to determine the viscosity of the liquid as given below.

$$\eta = \frac{d^2 g(\rho_s - \rho_l)}{18\mu} \quad \text{……..Equation 4}$$

Where η is viscosity, d is diameter of sphere, ρ_S is density of sphere, ρ_l is density of liquid, g is acceleration due to gravity and μ is terminal velocity.

Terminal velocity (μ) is determined when a liquid is placed in the fall tube which is clamped vertically in a constant temperature bath. Sufficient time must be allowed for temperature equilibration and for air bubbles to rise to the surface. A steel sphere is introduced into the fall tube through a narrow guide tube. The passage of the sphere is monitored and the time it takes to fall is recorded.

1.2.3.4.2 Capillary Viscometer

The rate of flow of the fluid through the capillary is measured under the influence of gravity or an externally applied pressure. Liquid is allowed to flow under gravity from a reservoir through a tube of known cross-section. In different instruments, the tube can vary from capillary size to a large diameter. The pressure difference across the ends of the tube and the time for a given quantity of flow are measured, and then the liquid viscosity for Newtonian fluids can be calculated as

$$C_v = \frac{1.25\Pi R^4 PT}{LV} \quad \text{.....Equation 5}$$

Where R is the radius (m) of the tube, L is its length (m), P is the pressure difference (N/m^2) across the ends and V is the volume of the liquid flowing in time T (m^3/s) (47).

1.2.3.4.3 Rotational Viscometer

The rotational viscometer involves concentric cylinder and cone and plate instruments. An approximation to uniform rate of shear throughout the sample is achieved by shearing a thin film of the liquid between concentric cylinders. The outer cylinder can be rotated or oscillated at a constant rate and the shear stress measured in terms of the deflection of the inner cylinder, which is suspended by a torsion wire or the inner cylinder can be rotated with the outer cylinder stationary and the resistance offered by the motor measured. This method is useful for studying the flow behavior of non-Newtonian liquids and highly viscous materials (48). The viscosity in poise (C_V) for Newtonian liquids is given by

$$C_v = 2.5G\frac{1/R_1^2 - 1/R_2^2}{\Pi h\omega}$$ ----------Equation 6

Where G is the couple formed by the torsion wire and its deflection, R_1 and R_2 are the radii of the inner and outer cylinders; h is the length of the cylinder and ω is the angular velocity (rads/s) of the rotating cylinder. (49)

1.2.4 Emulsion Components and Functionalities

1.2.4.1 Trifluoperazine

The IUPAC Chemical name of trifluoperazine is 10-[3-(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)-10H-phenothiazine. Trade names are Iatroneural®, Jatroneural®, Eskazinyl®, Eskazine®, Stelazine® and Terfluzine®. It is a white to off white crystalline powder with little or no odor (53, 54, 55). Trifluoperazine has surface

active properties. The critical micellar concentration (CMC) of trifluoperazine is 4.2 x 10^{-5}. The CMC increases with increasing pH, due to protonation of the second nitrogen atom of the drugs' piperazine ring (56).

Trifluoperazine hydrochloride is a phenothiazine antipsychotic used in the treatment of a variety of psychiatric disorders including schizophrenia severe anxiety, and disturbed behavior. It is also used for the control of nausea and vomiting. It is effective for the short-term treatment of generalized non-psychotic anxiety. Trifluoperazine is given as the hydrochloride but its doses are expressed in terms of the base. 1 mg of trifluoperazine is approximately equivalent to 1.2 mg of trifluoperazine hydrochloride (57).

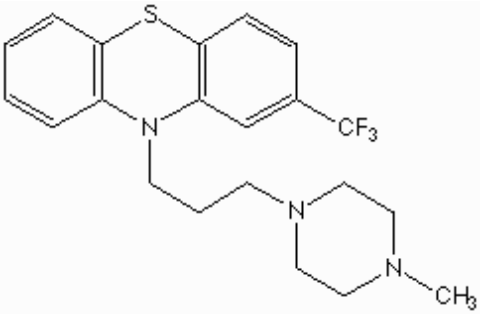

Figure 1. 3 Structure of Trifluoperazine

The usual initial dose for the treatment of schizophrenia and other psychoses is 2 to 5 mg twice daily by mouth, gradually increased to a usual range of 15 to 20 mg daily. In severe or resistant psychoses, daily doses of 40 mg or more have been given. For the control of acute psychotic symptoms it may be given by deep intramuscular injection in a

dose of 1 to 2 mg, repeated if necessary at intervals of not less than every 4 hours; more than 6 mg daily is rarely required. The initial dose for use in children is up to 5 mg daily by mouth in divided doses adjusted according to age, body-weight, and response, or 1 mg given once or twice daily by intramuscular injection (57).

For the control of nausea and vomiting, the usual adult dose by mouth is 1 or 2 mg twice daily; up to 6 mg daily may be given in divided doses. Children aged 3 to 5 years may be given up to 1 mg daily in divided doses; this may be increased to a maximum of 4 mg daily in children aged 6 to 12 years. When used as an adjunct in the short-term management of severe anxiety disorders, doses are similar to those used for the control of nausea and vomiting (55).

In recent studies trifluoperazine, a calmodulin antagonist has also been reported as effective in T-cell recovery of HIV patients. It binds to $Ca^{(2+)}$-calmodulin at high concentrations, it was found at low concentrations (10^{-6} to 10^{-10} M) to help T-cells from AIDS patients to restore proliferation in vitro (56, 57). Trifluoperazine also showed some significant antimicrobial activity. Forty six of 55 strains of Staphylococcus aureus were inhibited by 10–50 μg/ml of trifluoperazine. This drug also inhibited strains of Shigella species, Vibrio cholerae and Vibro parahaemolyticus at a concentration of 10–100 μg/ml. Other bacteria including Pseudomonas species were moderately sensitive to trifluoperazine (58). Preclinical studies have also shown that trifluoperazine can suppress opioid tolerance due to its inhibition of calcium/calmodulin dependent protein kinase II (59).

1.2.4.2 Soybean oil

Soybean oil is defined by the USP 26 as the refined fixed oil obtained from the seeds of the Soya plant Glycine soja (Leguminosae). In pharmaceutical preparations, soybean oil emulsions are primarily used as a fat source in total parenteral nutrition (TPN) regimens. Although other oils, such as peanut oil, have been used for this purpose, soybean oil is now preferred because it is associated with fewer adverse reactions (60). Soybean oil is used as an oleaginous solvent in the preparation of the oil-in-water emulsions. Fatty acid composition of soybean oil is shown in Table 1.1. It has a viscosity of 49mPa·s at 25°C.

Table 1.1 Fatty acid composition of soybean oil

Fatty acid	Percentage Composition
Palmitic acid	7-14%
Stearic acid	1-6%
Oleic acid	19-30%
Linoleic acid	44-62%

1.2.4.3 Tricaprylin

Tricaprylin is a medium chain triglyceride. Other names include glyceryl tricaprylate; trioctanoin; caprylic acid triglyceride; 1,2,3- trioctanoylglycerol. It is an odorless, colorless to light yellow liquid. Molecular weight of 470.69 g/mole.

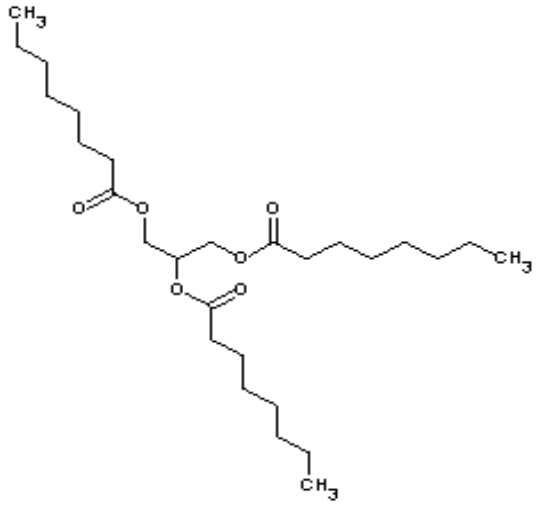

Figure 1. 4 Chemical Structure of Tricaprylin (61).

1.2.4.4 Tributyrin

Tributyrin is also known as glyceryl tributyrate. It is prepared by esterification of glycerol with excess butyric acid. Chemical abstract service (CAS) name is Butanoic acid 1,2,3-propanetriyl ester. Its molecular formula is $C_{15}H_{26}O_6$ and it has a molecular weight of 302.36. It is an oily liquid with bitter taste, insoluble in water but very soluble in alcohol and ether (62).

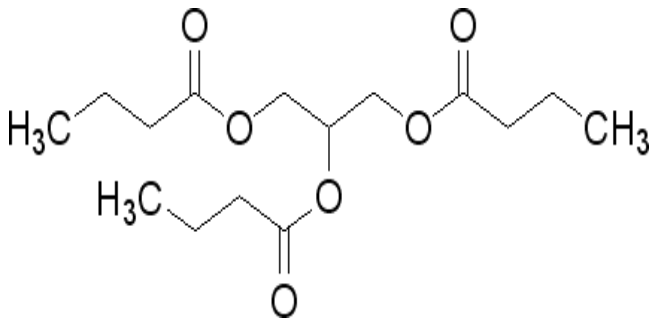

Figure 1. 5 Chemical Structure of Tributyrin

1.2.4.5 Ethyl Oleate

Other names include Ethyl 9-octadecenoate; oleic acid, ethyl ester. The chemical name is (Z)-9-octadecenoic acid, ethyl ester. The empirical formula is $C_{20}H_{38}0_2$. Ethyl oleate occurs as a pale yellow to almost colorless mobile oily liquid with a taste resembling that of olive oil. It is primarily used as a vehicle in parenteral preparations intended for intramuscular administration. It is a suitable solvent for steroids and other lipophilic drugs. It is less viscous than fixed oils and is more rapidly absorbed by body tissues (63).

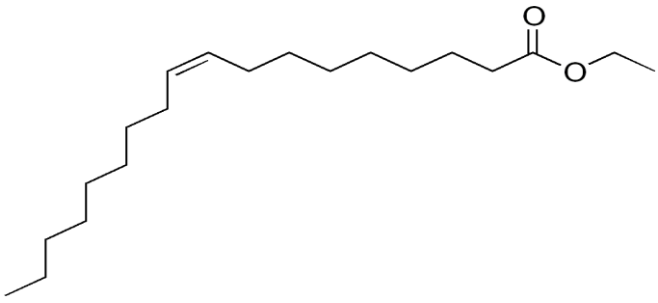

Figure 1. 6 Chemical Structure of Ethyl Oleate

1.2.4.6 Phosphatidyl choline

Phosphatidyl choline, also known as lecithin is a phospholipid which is a naturally occurring emulsifier obtained from either egg yolk or Soya beans. There several brands of lecithin available with different percentages of pure phosphatidyl choline. Hydrogenated egg phosphatidyl choline usually contain about 99% of phosphatidyl choline while soy derived phosphatidyl choline contains about 90%.

Phospholipids are biological surfactants with two hydrocarbon tails (the R groups) and a hydrophilic head (the phosphate salt group). Phosphatidyl choline is regarded as safe for parenteral use. It can be biodegraded and metabolized, since it is an integral part of biological membranes making it virtually non-toxic (64).

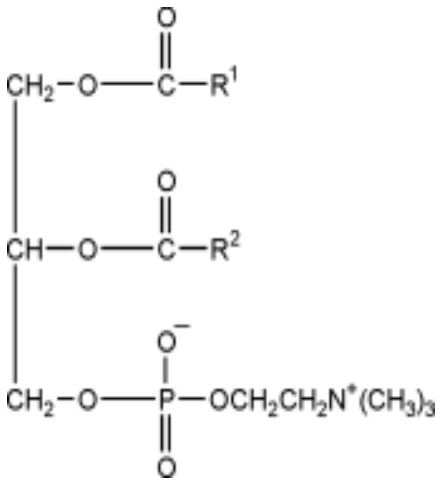

Figure 1. 7 α-phosphatidyl choline

(R_1 and R_2 = C_{15}-C_{17} hydrocarbon chains)

Phosphatidyl choline surfactant molecules have the ability to give necessary curvature of the interfacial film required to form nano-emulsions or microemulsions and this has been related to its packing geometry, which is the ratio of packing between the hydrocarbon volume, optimum head group area and tail length of the molecule at the surface. Phosphatidyl choline has a packing parameter around 0.8; this value is further increased if the oil phase penetrates into the alkyl chains of the lecithin molecule. In order to produce oil-in-water emulsions in the nano range, it is necessary to reduce this parameter by using co-surfactants or partial substitution of phosphatidyl choline by a more hydrophilic emulsifier (65).

1.2.4.7 Polysorbate 80

Polysorbate 80 (Chemical name: Polyoxyethylene 20 sorbitan monooleate, Chemical formula $C_{64}H_{124}O_{26}$) is a non-ionic surfactant also referred to as Tween 80. It is an oleate ester of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides. It has a HLB value of 15 and as a result is used in the production of oil-in-water emulsions (66).

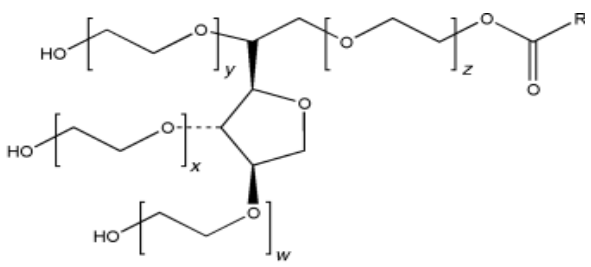

Figure 1. 8 Chemical Structure of Polyoxeyethylene sorbitan monoester
Polysorbate 80 (w + x + y + z = 80)

1.2.5 Statistical Experimental Design.

Experimental design can be defined as the strategy for setting up experiments in such a manner that the information required is obtained as efficiently and precisely as possible. Experimentation is carried out to determine the relationship between the factors acting on the system and the response or the properties of the system. This information was used to further and achieve the aims of the project (67).

Statistical tests provide the tools by which decisions can be made. Factorial designs are used in experiments where the effects of different factors, or conditions on experiments are to be elucidated (68). It is the design of choice for simultaneous determination of the effects of several factors and their interactions. Factorial designs have maximum efficiency in estimating main effects. If interaction exist, a factorial design is necessary to reveal and identify the interactions. Since factor effects are measured over varying levels of other factors, conclusions apply to a wide range of conditions. Maximum use is made of data since all main effects and interactions are independent of effect of other factors (69).

1.2.6 Stability Studies of Emulsions

The stability of any pharmaceutical product is defined as the capacity of the formulation to remain within defined limits for a predetermined period of time (shelf life of the product). The first step to designing any type of stability testing program is to specify these limits by establishing parameters defined in terms of chemical stability, physical stability and microbial stability. Next, methods must be established to evaluate these parameters (70).

Emulsions are thermodynamically unstable exhibiting flocculation and coalescence unless significant energetic barriers to droplet interactions are present. Emulsions are sensitive to coarsening phenomena like coalescence and Ostwald ripening since droplet size is not uniform and concentration gradients drive mass exchange, thus inducing changes in droplet sizes (71). Forced degradation studies involve subjection of emulsions to harsh conditions such as elevation of temperature. Emulsion characteristics are determined before and after the tests. As discussed in the experimental section, stability of emulsions were determined using the stress tests and accelerated stability studies of emulsions based on ICH guidelines were also determined. Stress tests included centrifugation and freeze thaw cycles of emulsions. Emulsion characteristics were determined before and after the tests.

1.2.6.1 Physical Instability

Instability of lipid emulsions can arise from changes in particle size of oil droplets leading to creaming and coalescence or from changes in pH, hydrolysis of emulsifier, or

oxidation of oil (72). There are basically 5 ways in which the structure of a dispersion of liquid droplets in a continuous medium can change resulting in physical instability.

1.2.6.1.1 Creaming

Creaming is the upward movement of dispersed droplets relative to the continuous phase. There is no change in droplet size, but buildup of an equilibrium droplet concentration gradient within the emulsion. This phenomenon occurs from external force fields, usually gravitational, centrifugal or electrostatic, acting on the system. The disperse phase, according to its density relative to that of the continuous phase, rises to the top or sinks to the bottom of the emulsion, forming a concentrated layer at the top of the emulsion. Sedimentation involves the same process but in the opposite direction (73).

1.2.6.1.2 Flocculation

In flocculation of emulsion droplets, there is no change in droplet size or distribution but the buildup of aggregates of droplets within the emulsion. Flocculation results from the existence of attractive forces between the droplets.

1.2.6.1.3 Coalescence

Emulsion droplets within a close- packed array resulting from sedimentation or creaming, coalesce to form larger droplets. This results in a change in the initial droplet size distribution.

1.2.6.1.4 Ostwald Ripening

Larger emulsion droplets are formed at the expense of the smaller droplets. In principle, the system will tend to an equilibrium state in which all the all the droplets attain the same size. Ostwald ripening is associated with the difference in chemical potential for emulsion of different size (74).

1.2.6.1.5 Phase Inversion

This is the process where an emulsion changes for example from an o/w emulsion to a w/o emulsion. This may be brought about by a change in temperature or concentration of one of the components or by the addition of a new component to the system (75, 76).

1.2.6.2 Chemical Instability

Chemical instability of an emulsion may occur as a result of drug degradation. Possible degradation pathways include hydrolysis, dehydration, isomerization and racemization, elimination, oxidation, photodegradation, and complex interactions with excipients and other drugs (77). The degradation pathway depends on the chemical nature of the drug. Trifluoperazine is a phenothiazine that is easily oxidized. It is subject to air and light induced oxidative degradation. The mechanism can be considered a two-step reaction involving the intermediate formation of a semiquinone free radical, which is then oxidized to the sulfoxide (78).

1.2.6.3 Regulatory Guidelines for Evaluation of Emulsion Stability

The International conference on harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use regulates the stability test by which emulsions and other drug products should be evaluated. This includes stress testing, long term, intermediate or accelerated stability studies.

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic instability of the molecule and validate the stability indicating power of the analytical procedures used.

ICH guidelines recommend stress test on a single batch of the drug substance. It should include the effect of temperatures (in 10°C increments (e.g., 50°C, 60°C, etc.) above that for accelerated testing), humidity (e.g., 75% RH or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photostability testing should be an integral part of stress testing (82).

ICH guidelines for accelerated stability studies includes testing at 40°C ± 2°C/75% RH± 5% RH for a minimum period of 6 months, intermediate studies at 30°C ± 2°C/65% RH ± 5% RH for at least 6 months and long term stability studies at 25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH for a period of 12 months on at least three primary batches (79).

2.0 Experimental

2.1 Materials

Soybean oil was purchased from Nacalai Tesque, Japan. Polysorbate 80 (Tween 80) was purchased from Spectrum Chemicals, New Brunswick, NJ.

Ethyl oleate, tricaprylin and tributyrin were also purchased from Spectrum chemicals. Hydrogenated egg phosphatidyl choline (HEPC) and Phospholipon 90® (Soybean lecithin) were donated by Avanti Polar Lipids, Alabaster, AL and Natterman Phospholipid GMBH, Germany respectively. Trifluoperazine hydrochloride was obtained from Spectrum Chemicals, NJ. HPLC grade acetonitrile, monobasic ammonium potassium phosphate and chloroform were obtained from Fisher Scientific, Pittsburgh, PA as shown in Table 2.1. A list of the equipments used for the experiments is shown in Table 2.2

2.2 Methodology

2.2.1 Preparation of Nano-emulsions

2.2.1.1 Thin Layer Hydration Method

Soybean oil, polysorbate 80 (Tween 80), phosphatidylcholine and the drug were accurately weighed into a 50ml flask. An appropriate volume of chloroform was added

to dissolve the materials in the flask. Chloroform was evaporated from the solution using a Buchi Rotavapor at a temperature of 35 degrees. After evaporation of solvent, the emulsion film was dried in a desiccator for at least 3 hours to remove any residual solvent. An appropriate volume of sterilized Millipore® water was added to the film flask using a disposable plastic syringe through a 0.22μm membrane filter. The flask opening was covered with a rubber closure. Two needles were inserted in to the rubber closure. Nitrogen gas was introduced into the glass through a rubber tube for 30 seconds to prevent oxidation of phosphatidyl choline. The nano-emulsion was formed through a series of sonication and vortex. This method has been previously described in literature (80).

2.2.2 Preliminary Emulsion Formulations

The preliminary experiments were screening experiments carried out prior to selection of emulsion components. Four different oil phases: soybean oil, ethyl oleate, tricaprylin and tributyrin were used in order to select the most suitable oil phase for the emulsion. Phosphatidyl choline was obtained from six different sources: soy lecithin, hydrogenated egg phosphatidyl choline, phospholipon 90 G, phospholipon 90 H, lipoid EPC and lipoid EPC-3 were used for the preliminary formulations (Table A1).

2.2.3 Optimization of Emulsion Components

After the preliminary emulsions were produced, more emulsions were formulated to determine the outcome of optimizing the concentration of the emulsion components.

The first set of emulsions were prepared in an Oil:Tween 80 weight ratio of 1:1, 2:1 and 3:1 at 9%, 16% and 23w/w% (oil/oil+water) soybean oil concentration. Emulsion composition is shown in Table 2.3. Each emulsion was replicated to validate the z-average, zeta potential and polydispersity index data. The effect of increasing concentration of phosphatidyl choline was evaluated and emulsion composition is shown in Table 2.4. The concentration of Tween 80 was also varied, formulation composition of emulsion is shown in Table 2.5. Drug content of emulsion was optimized by increasing trifluoperazine concentration and evaluating the outcome on emulsion characteristics, formulation composition is shown in Table 2.6. Phase diagrams were plotted to show the boundaries of the optimal formulation.

2.2.3 Z-average analysis, polydispersity index and zeta potential determination

Size, polydispersity index and zeta potential were simultaneously determined using the Zeta sizer® (Malvern instruments, Westborough, MA) and Nicomp380 ZLS® particle sizer (Santa Barbara, CA). Both instruments operate based on photon correlation spectroscopy. 1ml of Soybean emulsion was diluted to 50ml with Millipore water prior to z-average, zeta potential and polydispersity analysis. Disposable cuvettes were used.

Figure 2. 1 Branson® Sonicator

Figure 2. 2 Buchi Rotavapor®

Table 2.1 List of Materials

	Material	Manufacturer	Lot. no
1.	Polysorbate 80	Spectrum Chemical	TR0021
2.	Trifluoperazine HCl	Spectrum Chemical	SK0706
3.	Soybean Oil	Nacalai Tesque, Japan	M2G4769
4.	Phospholipon 90 H	Natterman Phospholipid GMBH	00250
5.	α-Phosphatidylcholine	Avanti Polar Lipids	830051P
6.	Lipoid EPC	Lipoid GMBH	105023
7	Lipoid EPC 3	Lipoid GMBH	276015-1
8	Phospholipon 90G	Natterman Phospholipid GMBH	10460
9.	Ammonium phosphate monobasic	Fisher Scientific, Pittsburgh, PA	954716
10.	Acetonitrile HPLC grade	Fisher Scientific, Pittsburgh, PA	954716
11.	Chloroform	Fisher Scientific, Pittsburgh, PA	973219
12.	Millipore water	Millipore corporation	

Table 2.1 List of Equipments

Equipment	Company	Model No	Serial No
Buchi Rotavapor	Brinkmann Instruments Inc Westbury, NY.	R-200	411713045011
Branson Sonicator	Branson Ultrasonics Corporation, Danbury, CT	2510R-DTH	RLC050384967
Shimadzu HPLC system	Shimadzu Scientific Instruments Inc., Columbia,	SCL-10A	20161E
Shimadzu HPLC Auto injector	Shimadzu Scientific Instruments Inc., Columbia,	SIL-10A	20218F
Shimadzu HPLC	Shimadzu Scientific Instruments Inc., Columbia,	SPD-10A	200936
Guard Column	Phenomenex, Torrance, CA	03A-0082-	2619281
Column	Phenomenex, Torrance, CA	Sphereclon	206197-3
Shimadzu HPLC	Shimadzu Scientific Instruments Inc., Columbia,	LC-10AS	20226A
Automated Microviscometer	ANTON PAAR, Ashland VA	AMVN	581875
Microcentrifuge	Denver Instrument Company		M005948
Equipment	Company	Model No	Serial No
Mechanical Convection Oven	Precision Scientific Inc.Chicago, IL	STM 80	11RU-11
Equipment	Company	Model No	Serial No
Nicomp Particle Sizer	Particle Sizing systems , Santa Barbara USA	3802LS	9805302
Malvern Zeta Sizer	Malvern Instruments	3000-HS	
Zeta sizer Nano ZS	Malvern Instruments	Nanoseries	
Fisher Scientific Low	Fisher Scientific, Pittsburgh, PA	307A	4100332
Denver Instruments Balance	Denver Instruments Company, Arvada, CO	AB-120	B044193
Denver Instruments Balance	Denver Instruments Company, Arvada, CO	400	0058954
pH Meter	VWR Scientific Products, West Chester, PA	9100	1013

Table 2.3 Formulation Composition: Effect of Surfactant to Oil Ratio

Oil:Tween 80 ratio	P.90 (mg)	Water (ml)	Trifluoperazine (mg)
1:1	250	10	40
2:1	250	10	40
3:1	250	10	40

Table 2.4 Formulation Composition: Effect of Phosphatidyl choline

Oil:Tween 80 ratio	P. 90 (mg)	Water (ml)	Trifluoperazine (mg)
1:1	100	10	40
1:1	250	10	40
1:1	500	10	40
1:1	750	10	40

Table 2.5 Formulation Composition: Effect of Tween 80

T.80 (mg)	P. 90 (mg)	Soy.Oil (mg)	Water (ml)	Trifluoperazine(mg)
300	250	1000	10	40
650	250	1000	10	40
1000	250	1000	10	40

Table 2.6 Effect of Drug Content

Oil:Tween 80 ratio	P.90 (mg)	Water (ml)	Trifluoperazine(mg)
1:1	250	10	40
1:1	250	10	60
1:1	250	10	80
1:1	250	10	120

2.2.4 Viscosity Determination

Dynamic and kinematic viscosities of the emulsions were determined using the Automated Microviscometer® (Anton Paar, Ashland, VA). The AMVn® is an automated high precision viscometer. It utilizes the rolling ball technique. Viscosity of emulsions were determined as shown in the diagram below (Figure 2.3) by observing the rolling time of a solid sphere under the influence of gravity in an inclined cylindrical tube filled with a sample liquid. The time taken by the ball to travel the fixed distance is measured with two inductive sensors. For each single rolling time, the result can be expressed as dynamic viscosity (mPa/s) and kinematic viscosity (mm^2/s) if sample density is known (81).

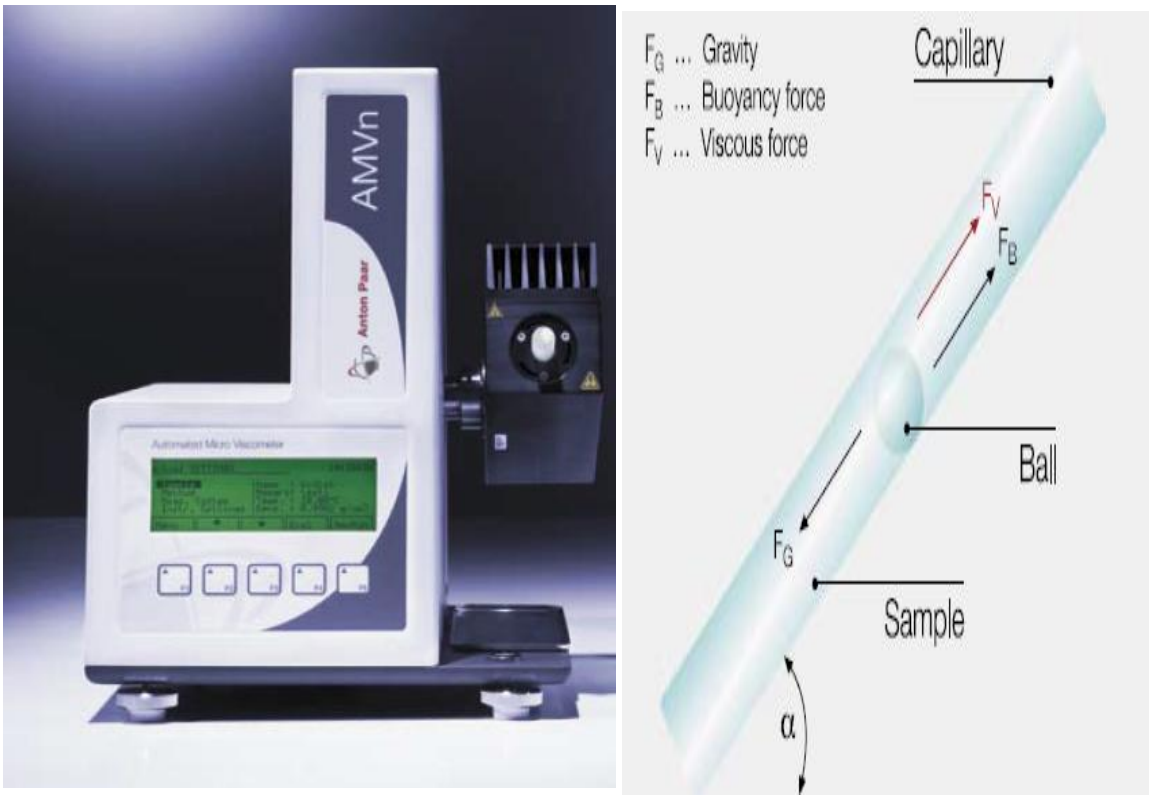

Figure 2.3 Automated Microviscometer (AMVn)

2.2.5 Ternary Phase Diagrams

In order to produce phase diagrams, over 50 different emulsions were prepared and characterized using the modified thin layer hydration method as previously described. The existence of nano-emulsion phase was identified as the area in the phase diagrams where clear and transparent formulations are obtained, based on visual inspection of many samples (82). Emulsions which had z-average less than 70nm and zeta potential greater than 25mV were selected as representative emulsions. These emulsions were selected because of transparence, relatively small z-average and high zeta potential, factors that indicate the potential stability of emulsions.

A phase diagram of representative emulsions (shown under the Results and Discussion section (Page 57) was plotted using JMP® software.

2.2.6. Statistical Design

The results obtained from the phase diagrams were used to develop a statistical experimental design. The phase diagram data showed the effect of different compositions of emulsion components on the emulsion characteristics. Based on these data, two levels were selected (high level and low level) for each component (factor) of the emulsion as shown in Table 2.7. A completely randomized 2X2X2X2X2 full factorial design experimental design was developed using JMP® software (Table 2.8).

This design enables testing of the main effects due to formulation factors of the emulsion, determination of the possibility of interactions within the model and the significance of these factors and the interaction terms. The factors were the emulsion

components such as Tween 80, soybean oil, Phospholipon 90G®, trifluoperazine and Millipore water. The dependent variables were z-average, zeta potential, polydispersity index, dynamic viscosity and kinematic viscosity of the emulsions. JMP® software was used to fit the model for each of the dependent variables. The p-values as shown under Results and Discussion (Page 59) indicate the level of significance of the factors. Pareto charts were plotted as graphical representations of the significance of the factors.

Table 2.7 Experimental Design Factors

Factors	Level	
	High	Low
Tween 80	1000mg	300mg
Phospholipon 90	750mg	250mg
Soybean Oil	3000mg	1000mg
Millipore Water	20ml	10ml
Trifluoperazine	120mg	40mg

2.2.7 High Performance Liquid Chromatography (HPLC) Analysis and Method Development

Several techniques have been utilized by researchers for HPLC analysis of phenothiazines. HPLC analysis is useful in determination of the stability of drug emulsions and identification of degradation products of trifluoperazine. A. Gindy et al. presented 3 methods for the determination of trifluoperazine in the presence of its degradation products (83). Many HPLC procedures have been described for the

determination of trifluoperazine singly or in combination with other phenothiazines (84-87) HPLC analysis was based on modification of a published method (87-90).

Table 2.8 2X2X2X2X2 Full Factorial Experimental Design

Experiment	T. 80 (mg)	P. 90 (mg)	Soy. Oil (mg)	Millipore water (ml)	Drug (mg)
1	300	750	3000	20	120
2	300	750	3000	10	40
3	1000	250	1000	20	40
4	300	250	1000	20	120
5	1000	750	3000	20	120
6	1000	750	1000	20	120
7	300	750	3000	20	40
8	300	750	1000	10	120
9	300	250	1000	20	40
10	1000	250	3000	20	120
11	1000	750	1000	10	40
12	300	250	1000	10	40
13	300	250	1000	10	120
14	1000	250	1000	20	120
15	300	250	3000	20	40
16	1000	250	3000	20	120
17	1000	250	1000	10	40
18	300	250	1000	10	120
19	1000	750	3000	10	120
20	300	250	3000	20	40
21	300	250	3000	10	120
22	300	750	1000	10	40
23	1000	750	1000	20	40
24	300	750	1000	20	40
25	1000	250	1000	10	120
26	300	750	1000	20	120
27	1000	250	3000	10	120
28	300	250	1000	10	40
29	300	250	3000	10	40
30	1000	750	1000	10	120
31	1000	750	1000	10	120
32	1000	750	3000	10	40

T.80= Tween 80, P.90= Phospholipon 90

2.2.7.1 HPLC conditions

Chromatographic analysis was carried out using a Shimadzu® HPLC system consisting of a Shimadzu auto injector SIL 10A®, Shimadzu system controller SCL-10A®, a Shimadzu SPD 10A UV® detector and a Shimadzu LC 10AS HPLC pump all from Shimadzu Scientific Instruments (Columbia, MD). Separations were carried out on a Sphereclone® C-18 column (150mm X 4.6mm with a particle size of 5µm) from Phenomenex (Torrance, CA) equipped with a guard column (30 X 4.6mm) also from Phenomenex (Torrance, CA). The wavelength of detection was 255nm and the flow rate was 1.5ml/min.

2.2.7.2 Preparation of Standard Solutions

Ten milligrams of trifluoperazine hydrochloride was dissolved in 100ml deionized water to produce a 100µg/ml solution. The mobile phase was acetonitrile: monobasic ammonium phosphate (60:40), adjusted with 0.2M phosphate buffer to pH 2.7.

2.2.7.3 Linearity and Range

Calibration curves were plotted using 1µg/ml-50µg/ml concentration range of trifluoperazine to determine linearity.

2.2.7.4 Precision and Accuracy

Precision and accuracy of the HPLC method were determined at 25%, 50% and 100% of the calibration curve range to test the validity of the method at low, mid and

high concentrations respectively. Triplicate samples were analyzed at each level. Precision was calculated as percent relative standard deviation (RSD) and accuracy was calculated as percent relative recovery. Inter-day variation was determined by repeating the procedure using independent samples on three separate days.

2.2.7.5 Drug Extraction

The emulsions were extracted and analyzed for drug content using HPLC analysis. Trifluoperazine HCl was extracted from the nano-emulsion prior to HPLC analysis using the following procedure: The extract was diluted with the mobile phase and agitated for >1hr. The diluted solution was passed through a 0.45μm filter. The extracted drug from the emulsion was then analyzed for the drug content using HPLC.

2.2.8 Emulsion Stability

2.2.8.1 Forced degradation Study

The optimal emulsion formulations Experiments 3 and 17 (Table 2.1) were prepared for the forced degradation studies. This experiment was designed determine the extent of emulsions degradation in terms of physical stability of the emulsions and the potency of the emulsions when subjected to high temperature over a period of 7 days. Blank emulsions were produced to compare with the drug loaded emulsions. Freshly prepared emulsions were stored in tightly sealed glass vials and placed in an oven at 65°C. Physical stability of emulsions were monitored by determination of z-averages, zeta

potentials and polydispersity indices of emulsions on days 0 & 7. Potency was monitored by analysis of drug content on days 0 & 7 using HPLC.

2.2.8.2 Stress Test

Several stress methods can be used to predict the stability of emulsions according to ICH guidelines (88). These include shaking, thermal cycling and freeze-thaw cycling. Emulsion droplet size distributions are then assessed (89). The stress tests of the emulsions included centrifugation and freeze/thaw cycle of the optimal emulsion formulations (experiments 3 & 17).

2.2.8.2.1 Centrifugation

Emulsions were centrifuged at 2000 X g/60min. The z-averages and zeta potentials of the emulsions were determined before and after centrifugation.

2.2.8.2.2 Freeze/thaw cycle

Emulsions were frozen for 24hours at -18°C and thawed at 25°C for another 24 hours. The z-averages and zeta potential values of the emulsions were determined before and after the freeze/thaw cycle.

2.2.8.3 Accelerated stability test

Optimal nano-emulsions of trifluoperazine: Emulsions 3 and 17 which have been shown to have z-averages less than 100nm were selected for stability studies at 40°C ±2°C at 75% relative humidity (RH) ±5%RH. These emulsions were stored sealed glass ampoules. Emulsion characteristics such as potency, z-average and zeta potential were analyzed on day 0 and once a month for a period of 3 months.

3.0 Results and Discussion

3.1 Preliminary Experiment Results

The preliminary experiments were screening experiments carried out for selection of emulsion components. Four different oil phases: soybean oil, ethyl oleate, tricaprylin and tributyrin were used in order to select the most suitable oil phase for the emulsion. Phosphatidyl choline was obtained from six different sources: soy lecithin, hydrogenated egg phosphatidyl choline, phospholipon 90 G, phospholipon 90 H, lipoid EPC and lipoid EPC-3 were used for the preliminary formulations.

Ethyl oleate emulsions had the lowest z-average while soybean oil emulsions had the highest z-average. Tributyrin emulsions had very large droplet sizes compared to the other formulations therefore were not further studied. Soybean oil emulsions had larger droplet sizes probably as a result of higher oil viscosity compared to ethyl oleate. (Figure 3.1) Soybean oil was selected as the oil phase despite the relatively higher emulsion droplet size because it has the least incidence of toxic reactions, greatest resistance to oxidation and widely used in research.

Blank emulsions (without drug) and drug loaded emulsions were produced. The incorporation of trifluoperazine into the formulation gave emulsions with considerably high zeta potential and low z-average sizes. Zeta potential of soybean oil emulsion was increased from 2.4mV (formulation 5) without drug to 43.2mV (formulation 9) in Table 3.1. This is because trifluoperazine confers a charge on the emulsion droplets. The

reduction of the z-average of emulsions can be attributed to the amphiphilic nature of trifluoperazine. Trifluoperazine is a cationic drug with surface active property and a critical micelle concentration (CMC) of 4.2 X $10^{-5.}$ The surfactant property should promote further lowering of surface tension by adsorption on the surface of oil droplets.

Glycerin was incorporated as a component of the emulsion to confer iso-osmocity based on the prototype formulation of Intralipid®. This was not further explored because a high polydispersity indices and relatively high z-averages were obtained. A different technique or modification of the method may produce smaller droplet sizes. Iso-osmocity may also be achieved using electrolytes.

The effect of the various sources of phosphatidyl choline was observed. Emulsions 11-25 were formulated using soy lecithin (Table 3.2). Concentrated emulsions were prepared by reducing the aqueous phase of the formulations. These emulsions (formulations 19-25) shown in Table 3.3 had high z-average. The increase in size may be due to reduced mobility as a result of the decreased aqueous phase. This possibly prevented collision that could yield smaller droplets. The zeta potentials of the emulsions were generally below 20mV thus indicating the potential instability of the emulsions. These emulsions had very large droplet sizes and may have exceeded the upper limit of detection of z-average for the Zeta sizer instrument, also the polydispersity indices at this point may not be reliable. Soy lecithin was not feasible in the production of nano-emulsions because of its oily, viscous nature.

Lipoid EPC, Lipoid EPC-3 and Phospholipon 90G® were utilized as source of phosphatidyl choline in formulations 26-28 (Table 3.4). Phospholipon 90 H was used for emulsions 29-31 (Table 3.5). Lipoid EPC® and lipoid EPC-3® are produced from egg

phosphatidyl choline while phospholipon 90® is produced from soybean phosphatidyl choline (90). Phospholipon 90 was selected as the phosphatidyl choline of choice because emulsion with low droplet size could be produced and polydispersity index was low.

The composition of the emulsion with the most desirable properties of low droplet size, low polydispersity index and high zeta potential is given in Table 3.6.

Emulsions with low z-average are usually more stable compared with emulsions with larger z-average because larger droplets are more susceptible to aggregation or creaming. As referred to under literature review, these phenomena are undesirable and can lead to a more serious problem of coalescence of the emulsion droplets (91). Due to the very small droplet size of the nano-emulsion there is a large reduction in the gravitational force and Brownian motion may be sufficient for overcoming the effect of gravity. In addition, smaller droplet size also prevents flocculation. Weak flocculation (cases where the net attractive forces are relatively weak) is prevented, thus enabling the system to remain dispersed with no separation (92).

Zeta potential measurement is a useful tool in the prediction of emulsions stability. A zeta potential larger than 25mV is typically required to stabilize a system (93).

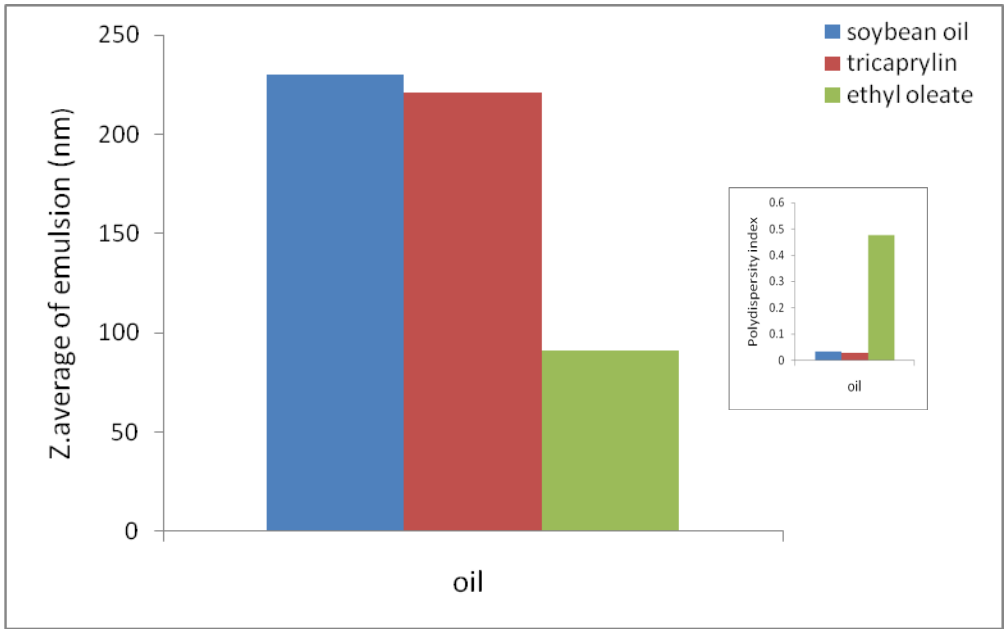

Figure 3.1 Effect different oil phases on Z-average and Polydispersity index of Emulsions

Table 3.1 Preliminary Emulsions

Form.	Oil (ml)	Tween80(ml)	HEPC (200mg)	Glycerin	Drug (mg)	Z.ave (nm)	P.I	Z.P (mV)
1	S.O	0.5	+	-	-	641.8	0.196	-12.4
2	E.O	0.5	+	-	-	2198.8	0.219	-12.9
3	S.O	0.5	+	+	-	374	0.84	-22.4
4	E.O	0.5	+	+	-	9932	0.806	-17.4
5	S.O	1	+	-	-	229.7	0.034	2.4
6	TRIC	1	+	-	-	221.1	0.030	2.8
7	TRIB	1	+	-	-	1329.4	0.165	18.4
8	E.O	1	+	-	-	91.4	0.477	2.5
9	S.O	1	+	-	40	118.5	0.54	43.2
10	TRIC.	1	+	-	40	139.2	0.3	32.5

Table 3. 1 Preliminary Experiments

Formulation	Tween 80	Oil	Soy. Lecithin	Drug (mg)	Water (ml)	Z.Ave (nm)	P. I	Z.P (mV)
11	1ml	1ml	200mg	-	10	3753.2	0.00	-64.1
12	1ml	1ml	200mg	40	10	41.3	0.00	11.8
13	1ml	1ml	200mg	40	10	8479.5	0.00	11.5
14	1ml	1ml	200mg	40	10	5297.7	0.00	9.4
15	10ml	10ml	2000mg	-	100	12485.6	0.002	-28.6
16	10ml	10ml	2000mg	400	100	10274.9	0.002	-0.1
17	300mg	1000mg	400mg	40	40	163.2	0.002	-21.5
18	750mg	1ml	250mg	40	40	696.4	0.002	9

Table 3. 2 Preliminary Experiments: Concentrated Emulsions

Formulation	Tween 80 (mg)	Oil (mg)	P.C (mg)	Drug (mg)	Water (ml)	Z.ave (nm)	P.I	Z. P (mV)
19	500	500	200	40	10	1347	0.002	19.7
20	500	500	200	40	6	1825.7	0.002	22.3
21	500	500	200	40	4	2099.7	0.002	22.8
22	500	500	200	40	2	554.3	0.002	20.6
23	500	500	400	40	10	825	0.002	-12.3
24	500	500	400	40	6	3533	0.002	-7.9
25	500	500	400	40	4	5218.4	0.002	-3.1

Table 3. 3 Emulsion Formulations Using 3 Different Sources of Phosphatidyl Choline

Formulation	Oil:Tween 80 ratio	Phosphatidyl choline	Drug (mg)	Water (ml)	Z.ave.(nm))	P.I	Z.P (mV)
26	1:1	Lipoid EPC	40	10	55	0.577	34.5
27	1:1	Phospholipon 90	40	10	39.4	0.293	32.2
28	1:1	Lipoid EP-3	40	10	153.8	0.372	32.9

Table 3. 4 Emulsions Formulation with increasing Phospholipon 90 H® content

Formulation	T.80 (mg)	P.90 (mg)	Soy.oil (mg)	Drug (mg)	Water (ml)	Z.ave.(nm))	P.I	Z.P (mV)
29	300	300	500	40	10	112.5	0.625	34.5
30	400	400	500	40	10	52.3	0.61	32.9
31	500	500	500	40	10	169.9	1	31.0

Table 3. 5 Emulsion Composition (Formulation 27)

Component	Amount
Soybean oil	1ml
Phosphatidyl Choline	200mg
Trifluoperazine	40mg
Water	10ml
Tween 80	1ml

3.2 Development of Optimal Formulations

In these series of experiments, emulsion characteristics such as z-average, polydispersity index, zeta potential were optimized by changing the concentration of the emulsion components.

The effect of soybean oil concentration on the mean particle size of emulsions was evaluated by increasing amount of soybean oil in a series of emulsions. The emulsions were produced in an Oil:Tween 80 weight ratio of 1:1, 2:1 and 3:1. (Table A2) When emulsions were prepared at 23w/w% (oil/oil+water) soybean oil concentration, the mean particle size was 136.2nm (Figure 3.2). The particle size fell along with the soybean oil concentration, at 9% w/w emulsions; the mean particle size was 34.2nm. The higher the oil phase ratio for an oil-in-water emulsion, the more viscous the emulsion since there is a greater number of oil droplets that require size reduction. This suggests that the oil constitutes the inner structure of the nano-emulsions, which is consistent with a direct o/w-type structure (94). The higher oil ratio also increases the interfacial tension between the aqueous phase and the oil phase, thus making it more difficult to form the emulsions. Polydispersity indices were generally low for all the emulsions, the highest being 0.46 at 23w/w%, while zeta potential of emulsions were not affected by the oil concentration.

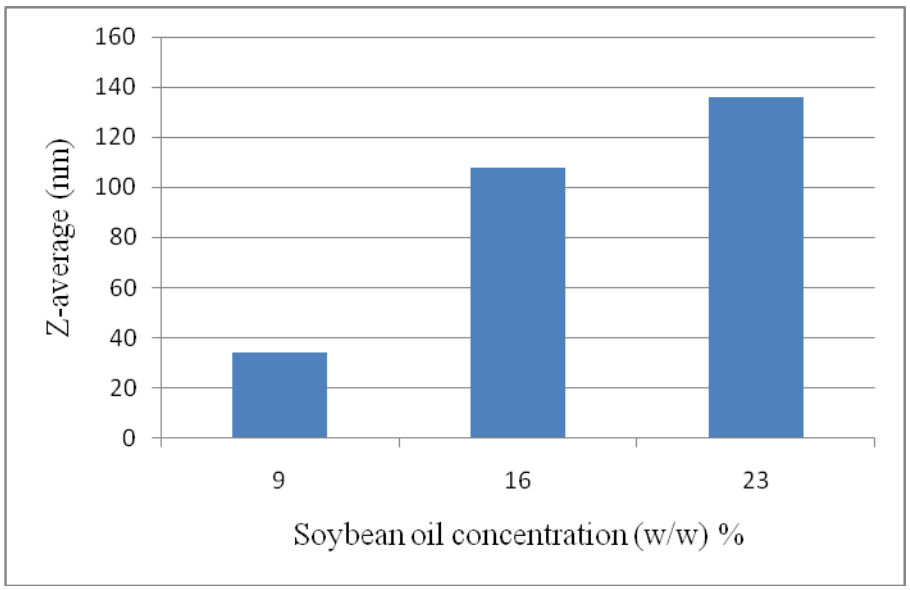

Figure 3.2 Effect of Soybean oil concentration on Z-average of Emulsions

The next step in the development of an optimal formulation was to determine the effect of Phospholipon 90G® on the z-average of the nano-emulsions. The emulsions were produced at 1:1 Soybean oil:Tween 80 with increasing concentration of Phospholipon 90G® at 0.9, 2.43,4.76,6.97 w/w% (Table A3). At high concentration of Phospholipon 90 G, the z-average of the emulsions were relatively high (Figure 3.3). This is in accordance with the HLB value of Phospholipon 90G ®. Phospholipon 90G® was used as a co-surfactant because of its ability to stabilize emulsion systems. However, it has lipophilic properties and a lower HLB of 9, therefore, at higher concentration it will tend to form a water-in-oil emulsion. There is also a tendency for Phospholipon 90G® to form emulsions that are more viscous and have a higher z-average at very high concentration. This is in accordance to Bancroft's rule, which states that the type of emulsion is dictated by the emulsifier and that the emulsifier should be soluble in the continuous phase. (Bancroft, 1913) (95,96). At high oil:surfactant ratio and high

Phospholipon 90G® concentration, the polydispersity indices of the emulsions also increased (Figure 3.4). High polydispersity index indicates that the emulsion droplet size are not uniform in the emulsions with large sizes.

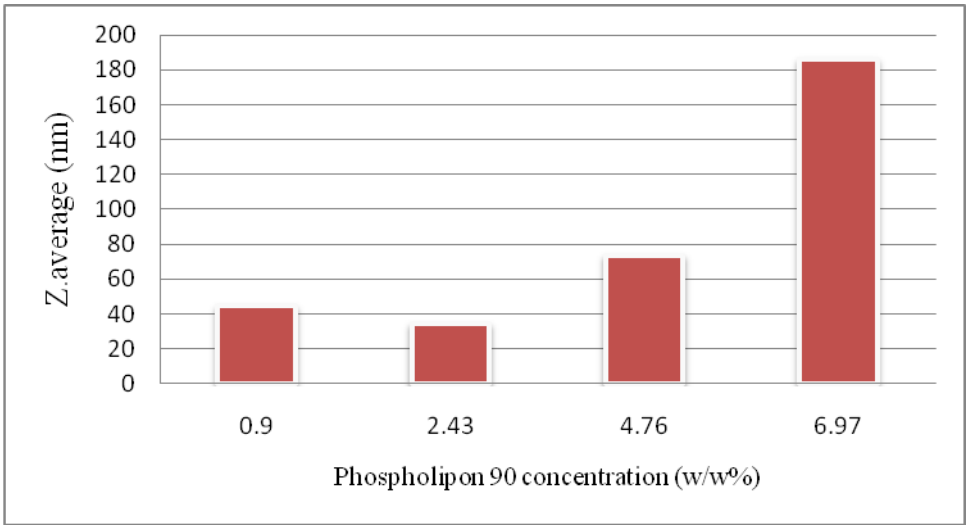

Figure 3.3 Effect of Phospholipon 90 on z-average of emulsions at 1:1 Soybean Oil:Tween 80 ratio

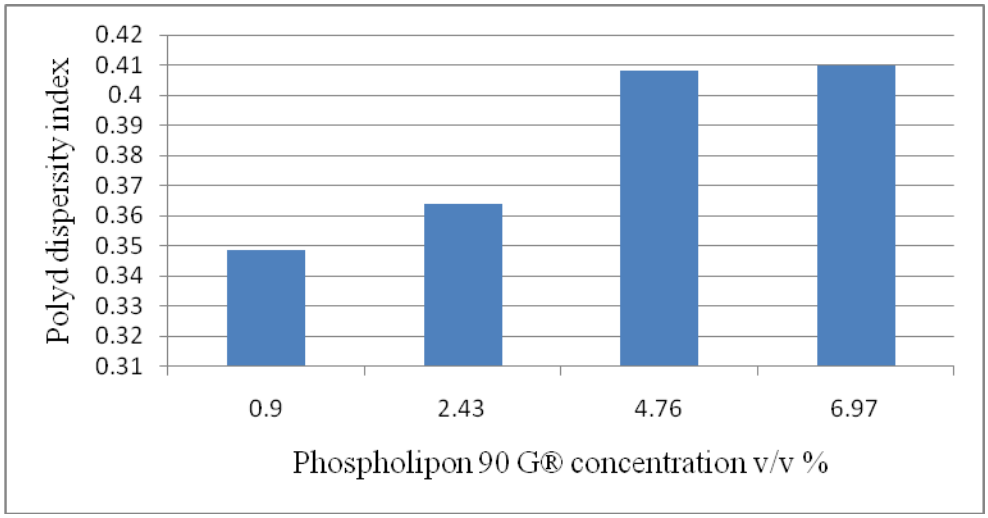

Figure 3.4 Effect of Phospholipon 90 on Polydispersity Index of emulsions at 1:1 Soybean Oil:Tween 80 ratio

The concentration of Tween 80 was increased while maintaining constant levels of other components of the emulsion (Table A4). It was observed that z-average of the emulsions reduced as the concentration of the surfactant increased (Figure 3.5). This is because the surfactant reduces the interfacial tension between the oil phase and the aqueous phase thus enhancing the formation of emulsions and reduction of z-average. The zeta potentials are generally about 30mV for all emulsions irrespective of Tween 80 concentration.

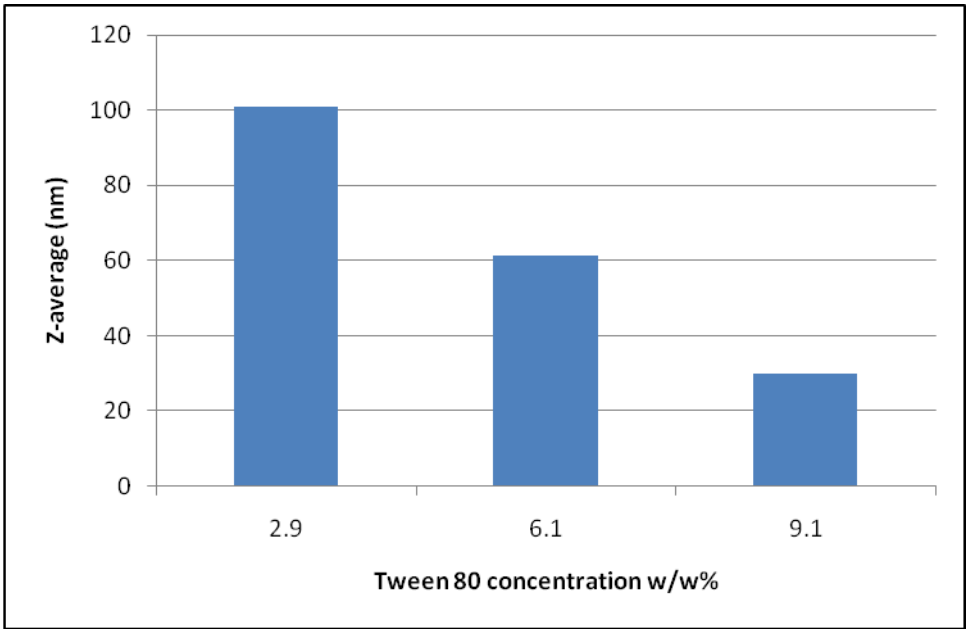

Figure 3.5 Effect of Tween 80 on Z. average of Emulsions

The drug content of emulsions was optimized by formulation of emulsions with increasing drug content. Drug loading of emulsions were at 0.4%, 0.6%, 0.8% and 1.2%w/v of trifluoperazine concentration. Emulsion droplet size were below 70nm indicating high concentration of drug was loaded. (Figure 3.6) Zeta potential of emulsions were in the acceptable range, above 25mV. There was an increase in zeta potential of emulsions at a higher concentration of trifluoperazine due to increased ionic strength of the emulsions. This is also an indication of potential stability. (Figure 3.7)

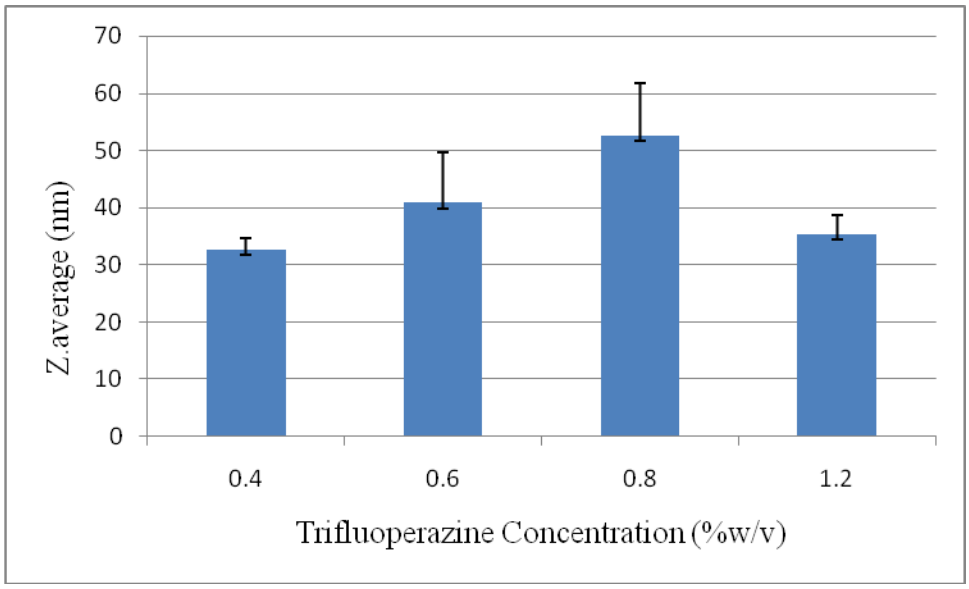

Figure 3.6 Effect of Optimization of Drug Content on Z.average Of Nano-emulsions

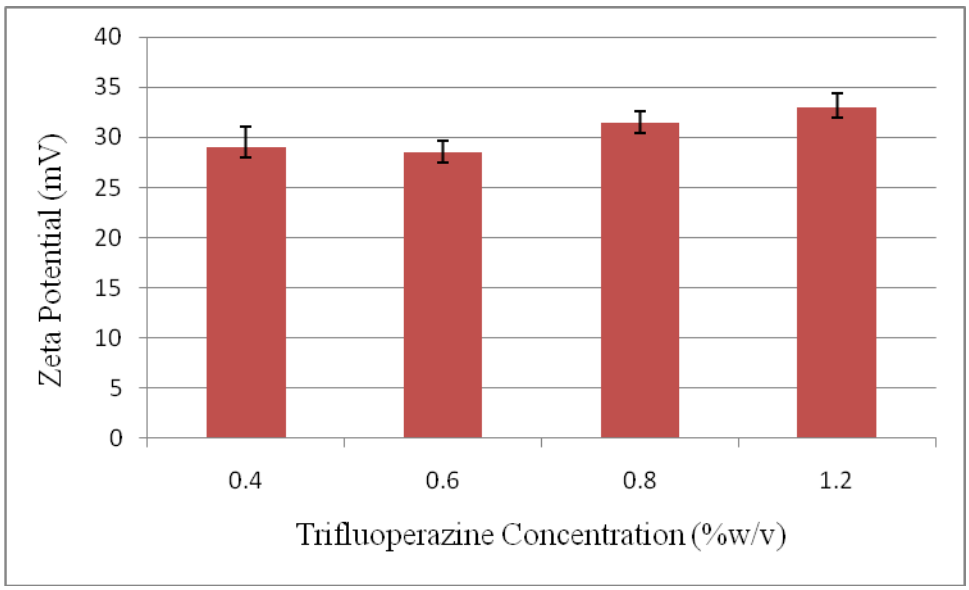

Figure 3.7 Effect of Optimization of Drug Content on Z. Potential Of Nano-emulsions

3.3 Phase Diagrams

A phase diagram indicating the region of the composition of the optimal formulations was plotted using JMP® software. In order to develop the phase diagram, components of the emulsions were varied to determine their effect on the emulsion characteristics.

From the previous experiments, representative emulsions were selected and shown in a phase diagram (Figure 3.8). The 3 different axes of the phase diagram represent the relative percentages of 3 components, the oil phase, surfactant and aqueous phase. The software normalizes the data so that all the components add up to 1. The shaded region within the phase diagram indicates the areas where optimum formulations

may be produced. Thirteen representative emulsions are shown in this diagram. The emulsions had relatively low z. average below 70nm and are also clear in appearance. These representative emulsions had low polydispersity indices of less than 0.5 and high zeta potentials, greater than 25mV.

From the phase diagram, we could identify the trends and relationships between surfactants, oil phase and the aqueous phase of the emulsions. The region producing the optimal formulation is shown to be that of high aqueous phase ratio, which is reasonable for the production of oil-in-water emulsions. The emulsions included in the phase diagram were emulsions with oil: surfactant ratio 1:1, the aqueous phase volumes were 10, 15 and 20mls and drug content of 40mg. A table of included emulsions is shown in Table A6.

Turbid emulsions were excluded in the phase diagram in Figure 3.8. Figure 3.9 shows all the emulsions in the experimental design. The emulsions with higher oil surfactant ratio can also be seen in the diagram. These emulsions had undesirable properties such as increased z-average, lower zeta potentials and were very turbid.

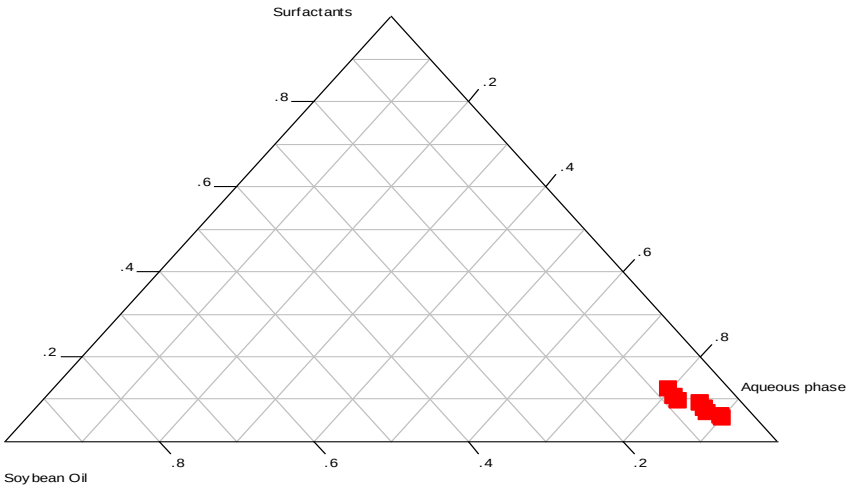

Figure 3.8 Ternary Phase diagram of Soybean emulsions showing region of optimal formulation

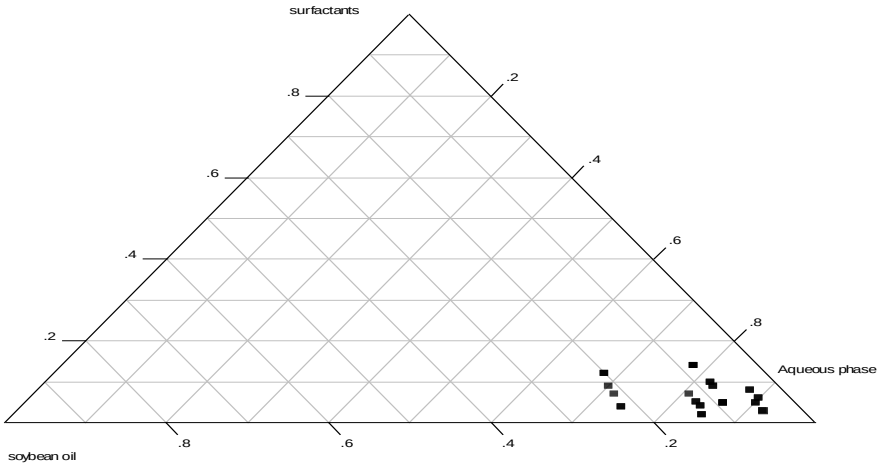

Figure 3.9 Ternary Phase diagram of Soybean emulsions showing experimental design formulations

3.4 Viscosity of Emulsions

The dynamic viscosity of emulsions with increasing levels of phosphatidyl choline was determined using the Automated Microviscometer (AMVn). These formulations and their compositions are shown in Table A7.

Phospholipon 90G® had a significant effect on the dynamic viscosity of the formulation. The dynamic viscosity increased significantly beyond 4% w/w of phosphatidyl choline content as shown in Figure 3.10. This is due to the nature of the surfactant. Phospholipon 90G® is more lipophilic and imparts a higher viscosity to the formulation. With increasing amount of the surfactant to oil ratio, an increase in the dynamic viscosity of these emulsions was observed. These data were obtained after dilution of emulsions at a ratio 1:50 with Millipore water. At high phospholipid levels for the pure undiluted emulsions as shown in Figure 3.10 the viscosity of emulsions increased when Phospholipon 90G® content was greater than 4%. The viscosity was extremely high when the phospholipid content was 750mg and at high surfactant levels to oil ratios.

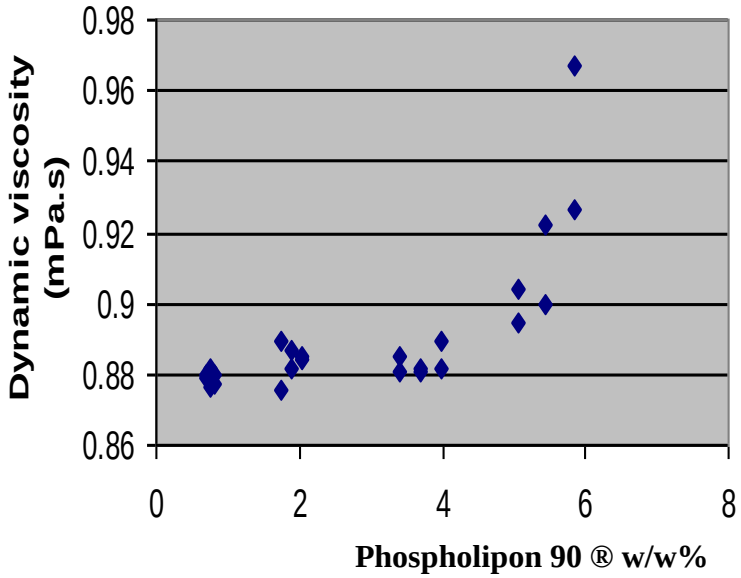

Figure 3.10 Effect of increasing %Phospholipon 90G™ on Dynamic Viscosity

3.5 Statistical Design of Experiments

JMP® software was used to design the experiment having identified the low and high levels of the formulation components as shown in Table 3.7. A completely randomized 2X2X2X2X2 full factorial design was used because it enables testing of the main effects due to factors (components) of the emulsion and determination of any interactions in the model, the significance of factors and elimination of bias. This yielded 32 experiments as shown in Table 3.8. The effects of the components of the emulsion on attributes such as z-average, polydispersity indices, zeta potential and viscosity were determined.

Analysis of variance (ANOVA) was used to determine the level of significance of the emulsion attributes and p-values were obtained using the "Fit Model" analysis in

JMP® software version 4.0. Parameter estimate values obtained from the analysis are given below in Table 3.9. Soybean oil showed the highest level of significance in determination of size based of the p-value of $\alpha < 0.0001$. For polydispersity index, soybean oil also showed significance at p-value of $\alpha = 0.0059$ and aqueous phase at $\alpha = 0.01$.For zeta potential, Tween 80 showed significance at $\alpha = 0.0249$ and Trifluoperazine at $\alpha = 0.01$. All emulsion components showed significance for dynamic viscosity.

Table 3.7 Levels of Emulsion Factors

Factors			Level	
			High	Low
X1	T80	Tween 80	1000mg	300mg
X2	P90	Phospholipon 90	750mg	250mg
X3	SO	Soybean Oil	3000mg	1000mg
X4	AP	Millipore Water	20ml	10ml
X5	TP	Trifluoperazine	120mg	40mg

The effect tests of the model were examined in order to determine the level of significance of the emulsion components on the Y values (z-average, polydispersity index, zeta potential and viscosity. The effects of interaction of any of the components, if present, were also determined in this statistical design. Pareto charts were plotted using JMP® software to show the variables and these were ordered according to their importance. The significance of any interactions is also shown.

Table 3.8 Statistical Design of Experiment Matrix

Exp.	T80 (mg)	P90 (mg)	SO (mg)	AP (ml)	TP (mg)	Size (nm)	PD	Z.P (mV)	Viscosity (mPa.S)
1	300	750	3000	20	120	127.7	0.184	39.86	2.1604
2	300	750	3000	10	40	157.2	0.145	44.61	8.7929
3	1000	250	1000	20	40	57.1	0.244	22.2	1.8145
4	300	250	1000	20	120	169.6	0.177	76.1	1.397
5	1000	750	3000	20	120	74.09	0.211	36.46	2.9118
6	1000	750	1000	20	120	73.47	0.271	34.34	2.6964
7	300	750	3000	20	40	144.2	0.182	28.66	2.3457
8	300	750	1000	10	120	91.71	0.223	32.92	5.2093
9	300	250	1000	20	40	93.56	0.176	27.68	1.4337
10	1000	250	3000	20	120	136.6	0.198	37.02	2.2481
11	1000	750	1000	10	40	88.95	0.264	28.58	15.9533
12	300	250	1000	10	40	97.23	0.201	29.85	2.1129
13	300	250	1000	10	120	90.12	0.193	37.67	2.0335
14	1000	250	1000	20	120	82.1	0.226	29.74	1.7702
15	300	250	3000	20	40	179.1	0.213	31.89	1.733
16	1000	250	3000	20	120	143.3	0.214	38.74	2.2214
17	1000	250	1000	10	40	72.97	0.225	25.59	3.91
18	300	250	1000	10	120	85.46	0.175	35.71	2.0271
19	1000	750	3000	10	120	154.8	0.168	37.28	9.2643
20	300	250	3000	20	40	190.1	0.236	39.64	1.7605
21	300	250	3000	10	120	183.8	0.219	43.15	3.2152
22	300	750	1000	10	40	81.92	0.213	29.64	5.1045
23	1000	750	1000	20	40	66.9	0.269	23.94	2.8091
24	300	750	1000	20	40	40.76	0.219	24.62	1.714
25	1000	250	1000	10	120	37.93	0.199	14.22	3.4661
26	300	750	1000	20	120	48.84	0.257	20.36	1.6605
27	1000	250	3000	10	120	145.4	0.18	33.67	5.5925
28	300	250	1000	10	40	90.33	0.187	33.63	1.9993
29	300	250	3000	10	40	181.6	0.169	36.78	3.0946
30	1000	750	1000	10	120	122	0.175	27.89	3.2214
31	1000	750	1000	10	120	71.21	0.242	30.31	11.3596
32	1000	750	3000	10	40	152	0.138	19.37	21.5565

3.5.1 Z-average Effects

Z-average effects and the level of significance of the individual factors as well as the interaction terms are shown in Figure 3.11. The concentration of soybean oil in the emulsion as shown in the pareto plot was the most significant factor in the determination of z-average of the emulsions. The significance of the oil phase in this statistical design further supports the effect observed in the phase diagrams that increasing the amount of soybean oil of the emulsions increased the z-average as shown previously in Figure 3.8. The interaction of the Phospholipon 90G® and the aqueous phase are seen to be the second most important factor based on the pareto plot in Figure 3.11. The Pareto plot shows the factors of the emulsions in order of importance with respect to the z-average of the emulsions.

3.5.2 Polydispersity Index Effects

The polydispersity index data and the level of significance of the individual factors are shown in Figure 3.12. The most significant factor was observed to be an interaction of Phospholipon 90G® and soybean oil. Other factors shown in decreasing order of importance are soybean oil, aqueous phase and Tween 80. The polydispersity of the emulsion shows the width of distribution of emulsion size, will be influenced by all these factors as shown in Figure 3.12.

3.5.3 Zeta Potential Effects

The zeta potential data and the level of significance of the individual factors are shown in Figure 3.13. The most important factor having significance on the zeta

potential of the emulsions is shown here to be the concentration of trifluoperazine in the emulsion. This is supported by the nature of the drug which confers a positive surface charge on the emulsion droplets. All other factors and interaction terms are shown in decreasing other of importance in the pareto plot.

3.5.4 Viscosity effects

The most important factor in the determination of viscosity was the aqueous phase of the emulsion as shown in the pareto chart (Figure 3.14). Phospholipon 90G® was a contributing factor and this confirms the previous findings of the influence of Phospholipon 90G® on viscosity of emulsions.

Graphs showing overlay plot of studentized residuals versus order of experiments were plotted for all the different Y variables. This data did not have any specific pattern thus indicating that there are no outliers in the data obtained. Studentized residuals did not indicate the presence of any outliers.

3.5.5 Optimal Emulsions

From the experimental design, emulsions 3 &17 were selected as best and optimal for consideration in the stability studies. The acceptable formulation criteria were: low z-average less than 100nm, low polydispersity indices <0.5 and relatively high zeta potentials >25mV.

Table 3. 9 Parameter Estimates of Size, Polydispersity Index, Zeta Potential and Dynamic Viscosity obtained from JMP® by ANOVA

Size				
Term	Estimate	Std Error	t Ratio	Prob>|t|
T80[300]	9.5947728	5.12255	1.87	0.0723
P90[250]	8.4748422	4.953124	1.71	0.0990
S0[1000]	-35.12499	5.027934	-6.99	<.0001
AP[10]	5.8354374	4.947362	1.18	0.2489
TP[40]	0.3127429	5.08468	0.06	0.9514
Polydispersity				
T80[300]	-0.008195	0.005362	-1.53	0.1385
P90[250]	-0.003302	0.005185	-0.64	0.5298
SO[1000]	0.0157765	0.005263	3.00	0.0059
AP[10]	-0.013549	0.005179	-2.62	0.0146
TP[40]	0.0013753	0.005323	0.26	0.7981
Zeta Potential				
T80[300]	4.1554993	1.745036	2.38	0.0249
P90[250]	1.6340967	1.687319	0.97	0.3417
SO[1000]	-2.356583	1.712804	-1.38	0.1806
AP[10]	-0.827518	1.685356	-0.49	0.6275
TP[40]	-3.952409	1.732135	-2.28	0.0309
Dynamic Viscosity				
T80[300]	-1.696946	0.552771	-3.07	0.0050
P90[250]	-1.796257	0.534489	-3.36	0.0024
SO[1000]	-1.006527	0.542561	-1.86	0.0749
AP[10]	2.2476529	0.533867	4.21	0.0003
TP[40]	1.143537	0.548685	2.08	0.0471

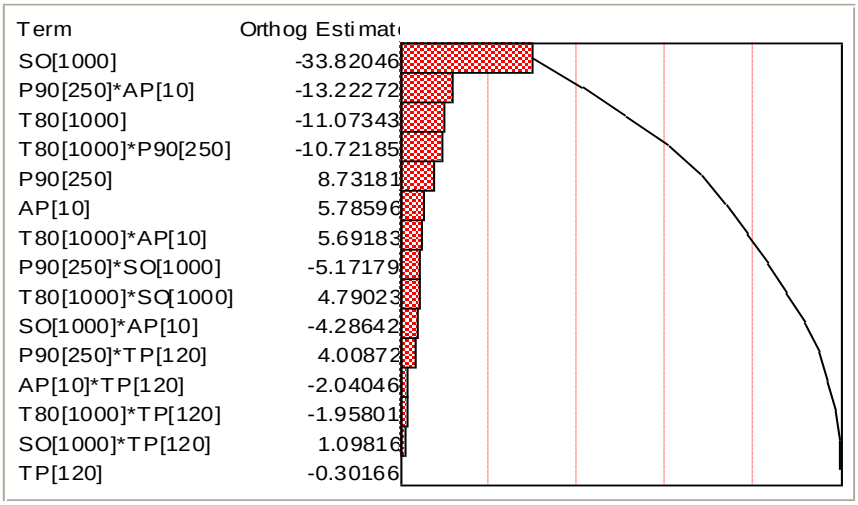

Figure 3.11 Pareto plot of Z-average Using Emulsions in the Statistical Design

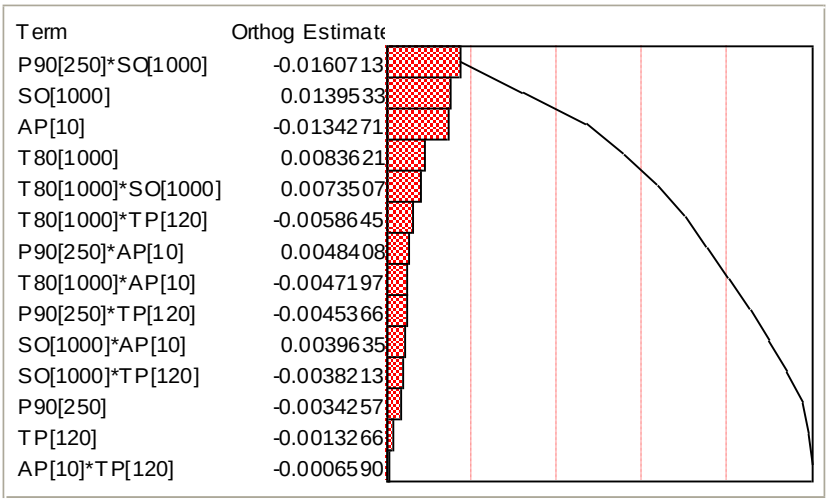

Figure 3.12 Pareto Plot of Transformed Estimates of Polydispersity index Using Emulsions in the Statistical Design

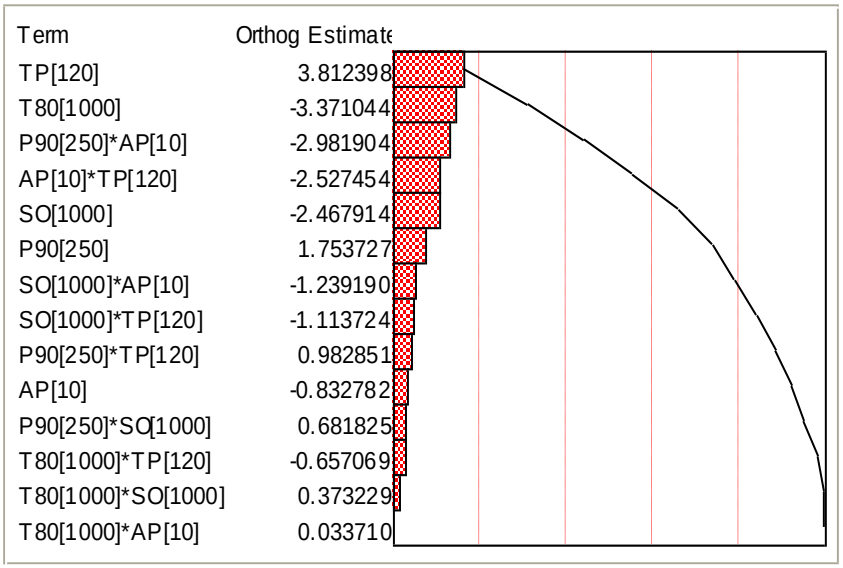

Figure 3.13 Pareto Plot of Transformed Estimates of Zeta potential Using Emulsions in the Statistical Design

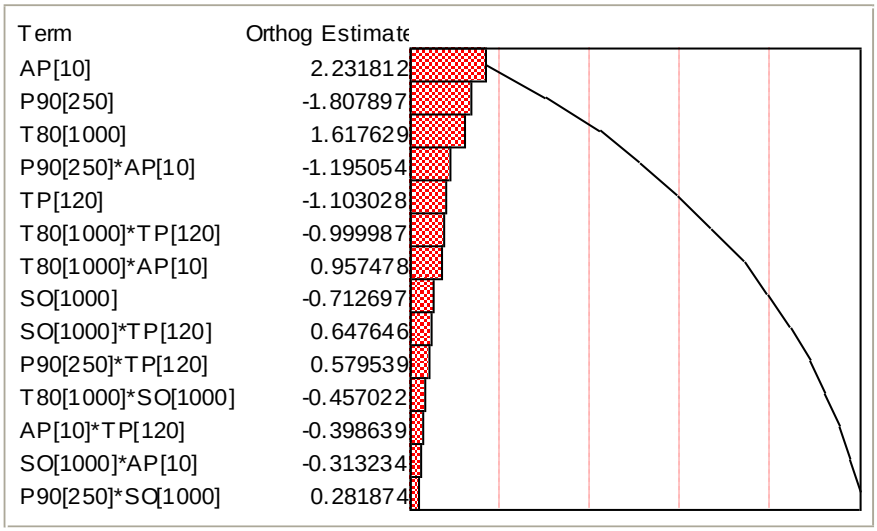

Figure 3.14 Pareto Plot of Transformed Estimates of Dynamic Viscosity Using Emulsions in the Statistical Design

3.6 HPLC Method Validation

Pure trifluoperazine was linear in the range 1-50µg/ml. The peak areas were analyzed and consistent and reproducible analysis was obtained. R-squared value of 0.9993 was obtained from the calibration curve (Figure 3.15). Inter-day accuracy of 97.97-101.3% with precision of 0.2-1.5% RSD was obtained from the calibration curve. Intra-day accuracy determined by analysis of variance (ANOVA) showed that at level of significance (α=0.05), p-value> 0.05 and Fcal < Fcrit, indicate no significant differences in the results between days or different concentration. Table A9.

Content analysis and % recovery were determined on the basis of the calibration curve

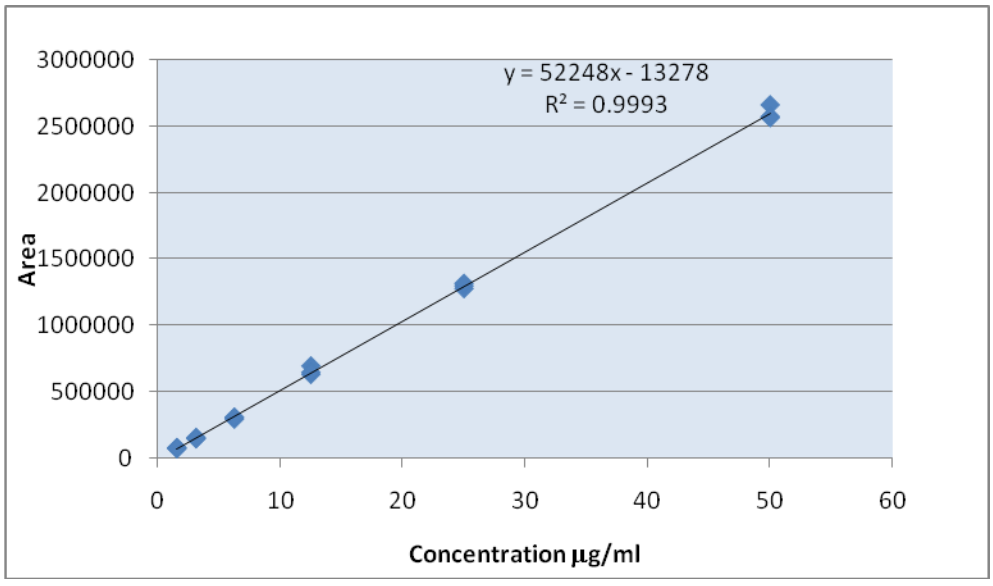

Figure 3.15 Calibration Curve for Trifluoperazine

3.7 Stability Studies of Optimal Formulations

3.7.1 Forced Degradation Studies

3.7.1.1 Z-average and zeta potential

The optimal emulsion formulations 3 & 17 were subjected to forced degradation for a period of 7 days. The z-average of the emulsions increased on storage at 65°C for both sets of emulsions. Z-average distribution immediately after preparation of the emulsion was increased from 44.0nm to 183nm and from 43.3nm to 133.5nm for emulsions 3& 17 respectively after storage for 7 days. Typical z-average distribution for emulsion 3 on day 0 is shown in Figure 3.15 and day 7 is shown in Figure 3.16. The z-average increased from about 36nm to over 100nm for the blank emulsions. The z-average of the optimal emulsions and blank emulsions increased due to aggregation of droplets because of the high thermal energy involved when stored at 65°C. This relatively high temperature increased the thermal energy of droplets and aggregation which eventually led to coalescence of droplets and eventual breakdown of emulsion.

The zeta potentials of both emulsions 3 & 17 increased after subjection to extreme temperature. In both cases, the zeta potential for the blank emulsion was very low since there are no active ingredients and therefore no charge. Increased zeta potential of optimal formulations can be attributed to breaking of the emulsions, thus more of the drug molecules are located at the interface of the emulsion droplets.

Table 3.10 Forced Degradation Test

	Emulsion 3		Blank Emulsion 3		Emulsion 17		Blank Emulsion 17	
	Z.Ave. (nm)	Z.P (mV)	Z.Ave. (nm)	Z.P (mV)	Z.Ave. (nm)	Z.P (mV)	Z.Ave. (nm)	Z.P (mV)
Day 0	44.0	14.86	36.0	2.34	43.3	16.45	37.5	1.93
Day 7	183.3	16.2	90.5	5.05	93.1	18.58	100.7	6.03

3.7.1.2 Potency

The drug content of emulsions 3 &17 were determined by HPLC. The emulsions were stored at 65°C for 7 days. Calibration curves were plotted for standards and potency was extrapolated from the curve. Drug content of emulsion 3 was reduced to 69.2 % after 7 days and potency of emulsion 17 was reduced to 72% after 7 days. Chromatograms of emulsions 3 on day 0 showed retention time of 8.5 minutes as shown in Figure 3.17 HPLC analysis showed emulsion degradation occurred. Figure 3.18 shows that the absorbance was reduced after 1 week of storage at 65°C. Blank emulsions containing no drug were included in the experiment to serve as control.

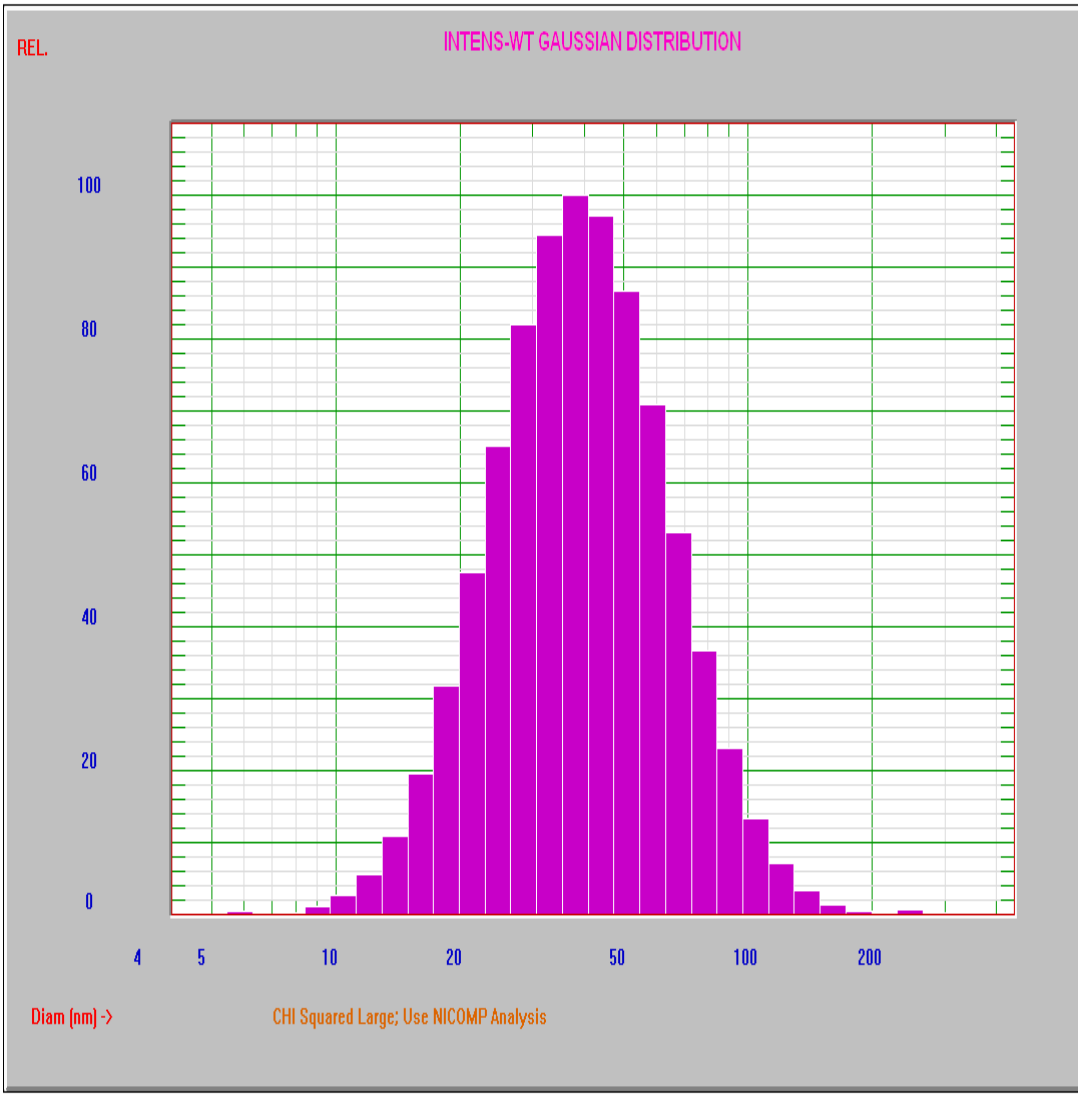

Figure 3.16 Z-average Distribution of Emulsion 3 at day 0

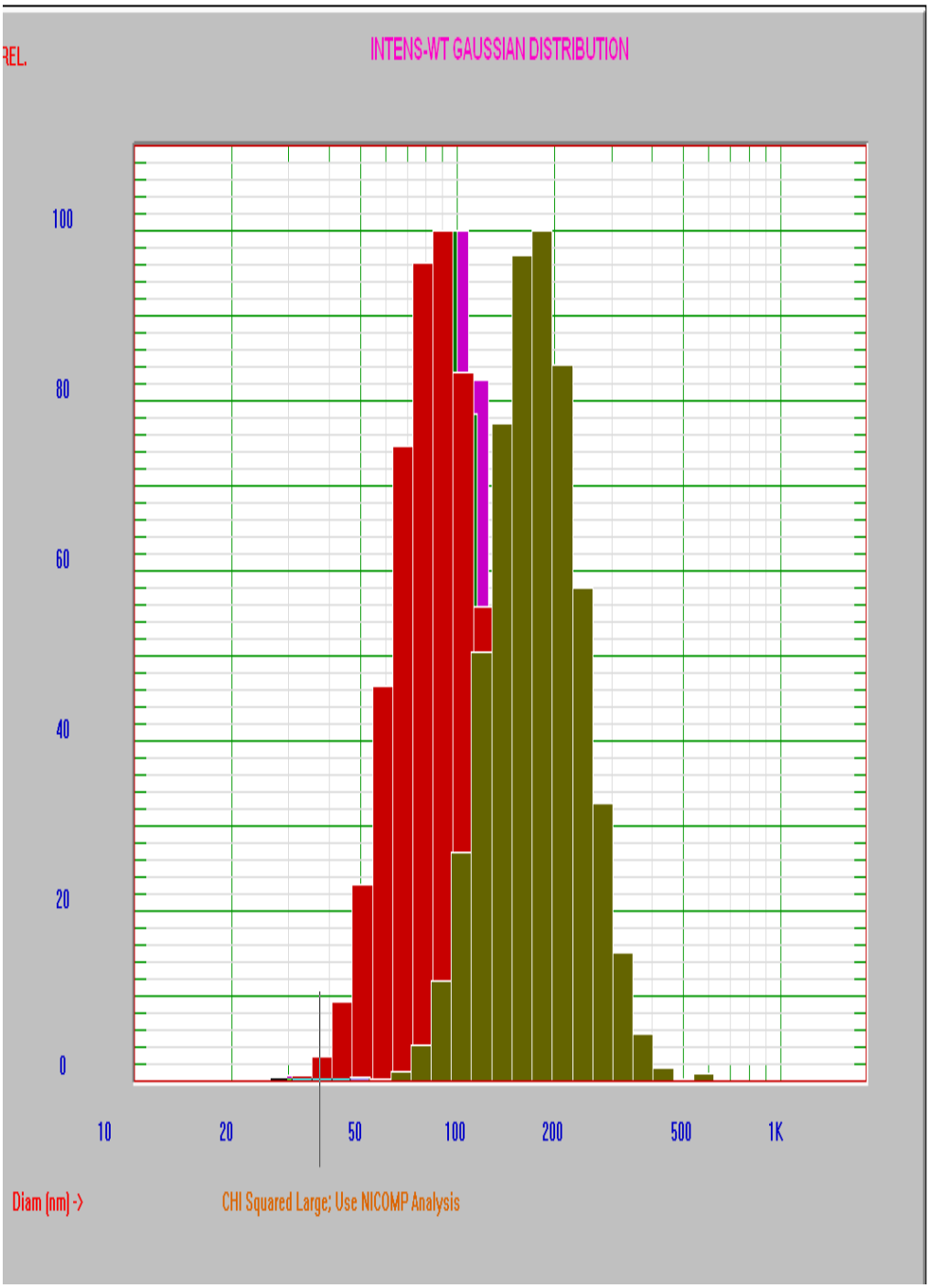

Figure 3.17 Z-average Distribution of Emulsion 3 after 1 week

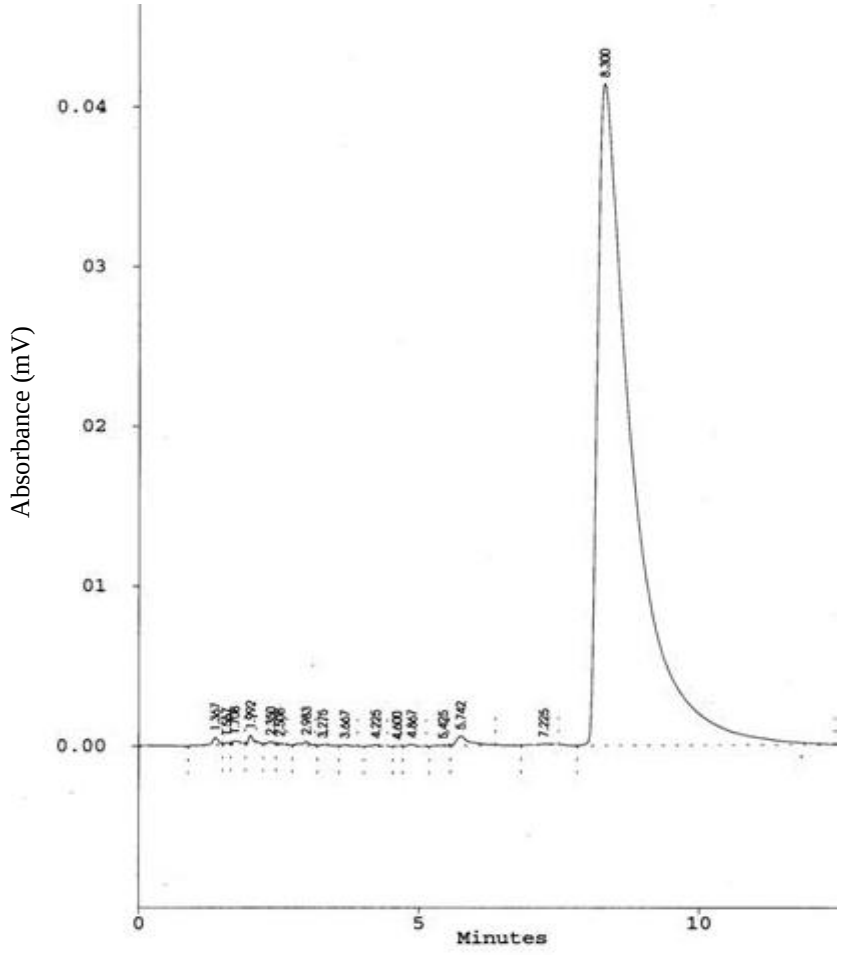

Figure 3.18 Typical Chromatogram at Day 0

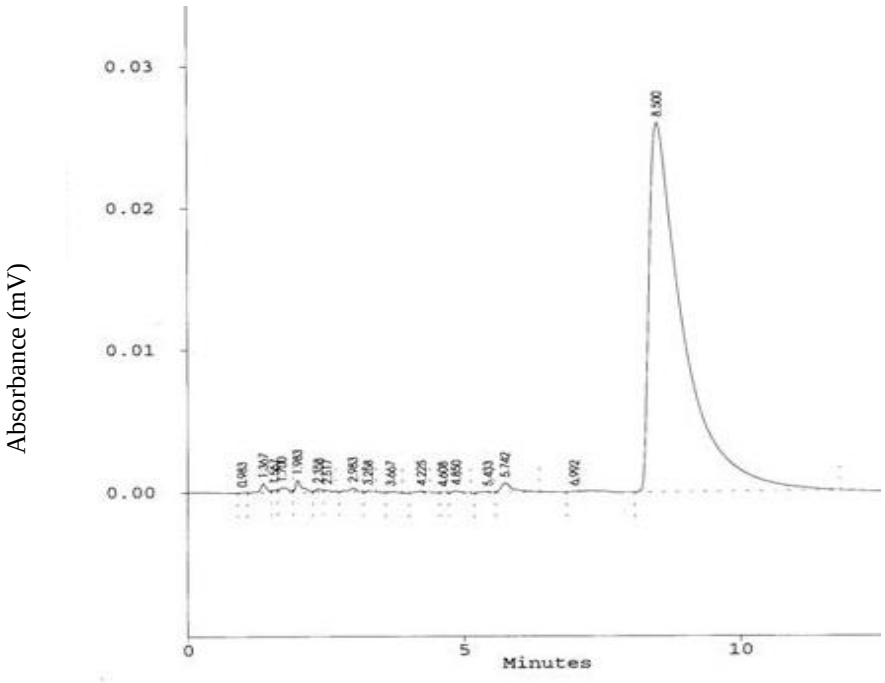

Figure 3.19 Typical Chromatogram of Emulsion stored at 65°C after 1 week

3.7.2 Stress test of Optimal Formulations

3.7.2.1 Centrifugation

Emulsions were centrifuged at 2000 X g/60min. Z-averages of emulsions were determined before and immediately after centrifugation. From the results obtained, it was observed that there were no significant changes in the z-average and the zeta potential of the emulsions before and after centrifugation on day 0 (Table 3.13).

3.7.2.2 Freeze/thaw cycle

Emulsions were frozen immediately after preparation for 24 hours at -18°C and thawed at 25°C for another 24 hours. The z-average values of the emulsions were

determined before and after the freeze/thaw cycle. All emulsions showed creaming after one freeze/thaw cycle process. A single cycle showed large increase in z-average of emulsion. Z-averages and the zeta potentials of emulsions increased considerably after the cycle. Emulsions 3 increased from 48.8nm to 243nm in z-average and Emulsion 17 increased from 51nm to 220.8nm. The zeta potentials of emulsions were observed to increase after the freeze thaw cycle. Destabilization of emulsion was greater with the freeze-thaw cycle than centrifugation. Majority of oil droplets were compressed together between the advancing ice crystals as the continuous phase of the emulsion freezes and it is not surprising that most of the oil droplets have coalesced (97). This is in contrast to centrifugation which is characterized by an insignificant change in z-average and zeta potential of emulsions.

Table 3. 11 Stress Test

Experiment	Stress Test	Z-average (nm)		Zeta Potential (mV)	
		Before	After	Before	After
Emulsion 3	Centrifugation	48.89	48.99	21.1	23.8
	Freeze/Thaw	48.89	243.5	21.1	32.7
Emulsion 17	Centrifugation	51.56	52.33	20.5	20.8
	Freeze/Thaw	51.56	220.8	20.5	34.6

3.7.3 Accelerated Stability Test

Accelerated stability test of selected Soybean emulsions of trifluoperazine was developed according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines. The guideline recommends storage at 40°C ±2°C at 75% relative humidity (RH) ±5%RH. A 6-month study is recommended for an accelerated stability study. However, if significant change occurs within the first 3 months it is considered unnecessary to continue to test through 6 months (98).

Emulsions 3 and 17 shown in the statistical design were reproduced in triplicates and used for the accelerated stability study. Emulsions were stored at 40ºC±2/ 75 RH ±5% RH. Drug content analysis of emulsions using HPLC, z-average and zeta potential of the emulsions were determined over 3 months.

3.7.3.1 Content Analysis of Emulsions 3 and 17

Drug content of Emulsions 3 & 17 was determined on day 0 by HPLC after extraction of pure drug from emulsions by agitation for 1 hour in the mobile phase. The emulsions were filtered using 0.45µm filter and then analyzed by HPLC.

Emulsions were stored in glass ampoules at 40ºC±2/75 RH ±5% RH, room. Drug content analyses of emulsions were determined after 1 month, 2 months and at 3 months (Table A10). These emulsions showed less than 75% potency after 3 months of storage .

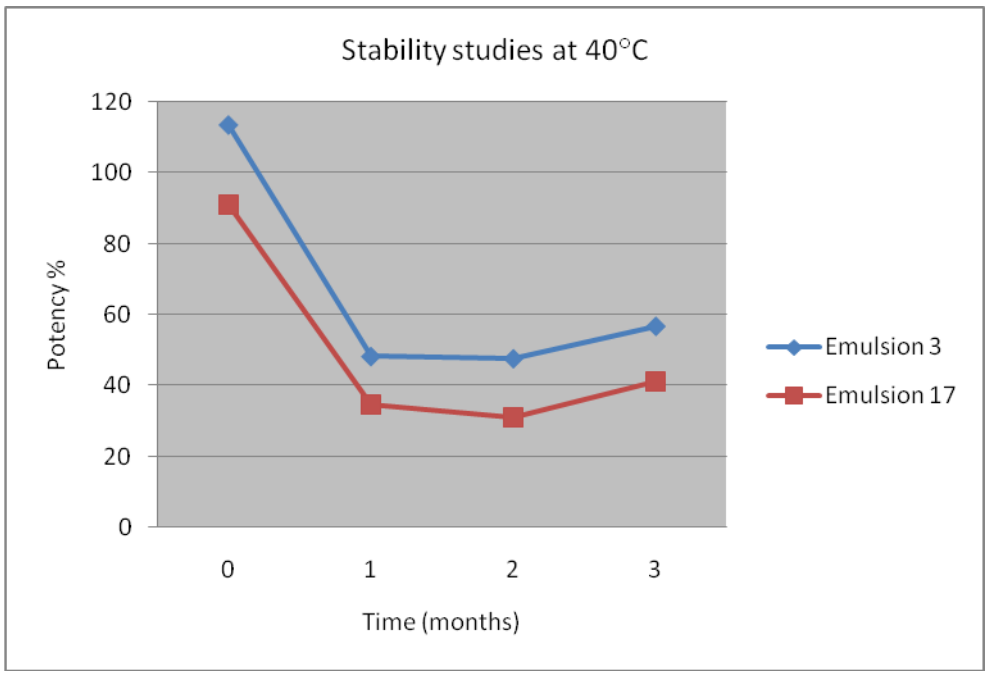

Figure 3.20 HPLC Analysis of Emulsions 3 & 17 at 40ºC/ 75RH

Emulsion breakdown can occur through physical degradation such as flocculation, Ostwald ripening etc. as discussed previously in the literature review and chemical degradation due to the instability of the drug. Factors responsible for the degradation of trifluoperazine were due to temperature, pH, air and light induced oxidation. The most likely degradation pathway for trifluoperazine is as shown in the figure below. Oxidation of trifluoperazine occurs through a two-step reaction involving the intermediate formation of a semiquinone free radical which is then oxidized to the sulfoxide.

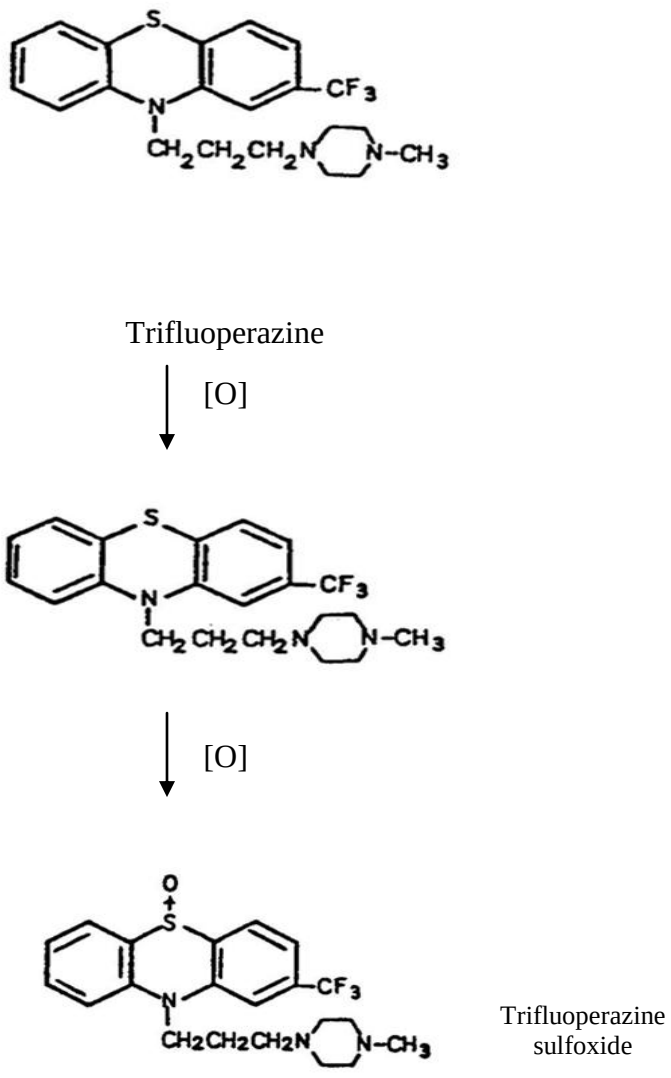

Figure 3.21 Degradation Pathway of Trifluoperazine

3.7.3.2 Z-average analysis and Zeta Potential of Emulsions 3 &17 after 3 months storage *at 40°C/75% RH*

Z-average results were determined using the Nicomp particle sizer after 1, 2 &3 months of storage at 40ºC/75 RH. Emulsion 3 had a z-average of 45.3nm immediately after preparation. Z- average value for Emulsion 3 was 61.4nm at 40°C after 3 months. The z-average of Emulsion 17 also increased from 21.3nm at day 0 to over 101.7 nm after 3 months at 40°C/75RH.

Zeta potential value for Emulsion 3 was 17.34mV on day 0, this reduced drastically to 3.97 mV at 40°C (Table 3.12). Zeta potential of Emulsion 17 on day 0 was 21.32mV; this decreased to 6.36mV and 3.97mV after 3 months at 40ºC/75%RH.

Table 3.12 Accelerated Stability Tests showing Z. average and Zeta potential Data For Z-average and Zeta Potential of Emulsions 3 & 17 Stored at 40ºC and 75RH

	Z-average (nm)		Zeta potential (mV)	
Emulsion	3	17	3	17
DAY 0	45.3	21.3	17.34	21.32
1 MONTH	71.3	93.9	14.1	5.27
2 MONTHS	67.3	126.5	10.82	3.63
3 MONTHS	61.4	101.7	7.5	3.97

3.8 Summary

This research study describes a novel method for the preparation and characterization of emulsions intended for parenteral delivery using the thin layer hydration method. It is a very versatile method for producing nano-emulsions.

Oil-in-water nano-emulsions of trifluoperazine for parenteral delivery were produced using soybean oil and a blend of surfactants; Tween 80 and Phospholipon 90G®. This was developed with the intention to provide a system which is more stable and better formulated to possibly prevent adherence to PVC tubing.

3.8.1 Emulsion Formulation and Characterization

Emulsions were formulated and characterized to determine z-average, polydispersity, zeta potential and viscosity. The emulsion components such as soybean oil, Tween 80, Phospholipon 90G®, Millipore water and trifluoperazine were varied to produce an optimal formulation. The oil: surfactant ratio was a very important factor in the determination of the z-average of the emulsions. Phase diagrams were produced following the preliminary emulsions and this showed the best formulation parameter ranges for emulsion formulation. A 2X2X2X2X2 factorial statistical experiment was designed to test the significance of the emulsion components and determine the influence of various emulsion components on the emulsion characteristics. Pareto charts were plotted to evaluate the significance of the factors and the presence of interactions if any.

Emulsions with z. averages of less than 100 nm were obtained. The small z-average of the emulsions suggests that emulsions will be stable. They also had polydispersity indices of less than 0.4. Zeta potentials were greater than 20 mV indicating potential stability of emulsions. Viscosity data was obtained for emulsions. Viscosity data can be exploited as index of emulsion stability if changes are observed in the emulsion viscosity with time.

3.8.2 Stability and HPLC Analysis

Emulsions were analyzed using HPLC immediately after production to determine drug content. Stability tests were done at 40ºC/75 RH to determine the level of degradation according ICH guidelines. The emulsion droplet sizes were not significantly increased thus indicating that the method is useful in formation of emulsions that can maintain integrity without breaking or coalescing. The drug content of emulsions was observed to reduce on storage mainly as a result of chemical degradation of the drug.

The success of an emulsion as a drug product is dependent on the z-average, the zeta potential also the characteristics of the drug in question. The chemistry of a drug plays a large role in the determination of the zeta potential and how quickly a drug will be degraded. Other components may be included in the formulation to ensure stability of drug for further development. This method is very useful in the initial development process of drugs to be formulated as emulsions.

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5.0 Appendix

Table A 1 Preliminary Emulsions

Formulation	Oil phase	Oil Phase	Tween 80	Phosphatidyl choline	Drug (mg)
1	S.O		0.5	200mg	0
2	E.O		0.5	200mg	0
3	S.O		0.5	200mg	0
4	E.O		0.5	200mg	0
5	S.O		1	200mg	0
6	TRIC		1	200mg	0
7	TRIB		1	200mg	0
8	E.O		1	200mg	0
9	S.O		1	200mg	40
10	TRIC.		1	200mg	40
11	S.O	1ml	1ml	200mg)	-
12	S.O	1ml	1ml	200mg	40
13	S.O	1ml	1ml	200mg	40
14	S.O	1ml	1ml	200mg	40
15	S.O	10ml	10ml	2000mg	-
16	S.O	10ml	10ml	2000mg	400
17	S.O	300mg	1000mg	400mg	40
18	S.O	750	1ml	250mg	40mg
19	S.O	500mg	500mg	200mg	40mg
20	S.O	500mg	500mg	200mg	40mg
21	S.O	500mg	500mg	200mg	40mg
22	S.O	500mg	500mg	200mg	40mg
23	S.O	500mg	500mg	400mg	40mg
24	S.O	500mg	500mg	400mg	40mg
25	S.O	500mg	500mg	400mg	40mg
26	S.O	1ml	1ml	Lipoid EPC (250mg)	40mg
27	S.O	1ml	1ml	Phospholipon 90	40mg
28	S.O	1ml	1ml	LipoidEP-3 (250mg)	40mg
29	S.O	500mg	300mg	300mg	40mg
30	S.O	500mg	400mg	400mg	40mg
31	S.O	500mg	500mg	500mg	40mg

Table A 2. Effect of Increasing Oil: Surfactant ratios on Z. average, polydispersity and zeta potential

Formulation	Tween 80	Oil: Surfactant ratio	Water	Soy. oil	P. 90	Z.Ave (nm)	P.I	Z.P (mV)
32a	1000	1:1	10ml	1000	250mg	32.9	0.316	30.4
32b	1000	1:1	10ml	1000	250mg	35.5	0.412	29.7
33a	1000	2:1	10ml	2000	250mg	110.7	0.385	30.6
33b	1000	2:1	10ml	2000	250mg	105.3	0.303	28.6
34a	1000	3:1	10ml	3000	250mg	123.4	0.509	30.0
34b	1000	3:1	10ml	3000	250mg	149.0	0.420	31.9

Table A 3. Effect of phosphatidylcholine on z-average, polydispersityindex and zeta potential (Soybean oil: Tween 80 1:1)

Form.	T.80 (mg)	Soy. Oil (mg)	Water (ml)	P.90 (mg)	Z.Ave (nm)	P.I	Z.P (mV
35a	1000	1000	10	750	187.1	0.405	27.1
35b	1000	1000	10	750	184.6	0.415	37.8
36a	1000	1000	10	500	65.0	0.418	32.7
36b	1000	1000	10	500	81.5	0.398	26.6
32a	1000	1000	10	250	32.9	0.316	30.4
32b	1000	1000	10	250	35.5	0.412	29.7
37a	1000	1000	10	100	44.1	0.340	35.9
37b	1000	1000	10	100	44.6	0.357	31.8

Table A4. Effect of increasing amount of Tween 80 on Average of Emulsions

Form.	Tween 80 (mg)	Z-average (nm)
52a	300	97.2
52b	300	104.7
53a	650	67.3
53b	650	55.4
54a	1000	30.1
54b	1000	29.9

Table A5. Emulsion formulations varying the concentration of Trifluoperazine

Form.	T.80 (mg)	Soy. oil (mg)	Drug (mg)	Water (ml)	P. 90 (mg)	Z. Ave. (nm)	P.I	Z.P (mV)
54a	1000	1000	40	10	250	30.1	0.288	29.6
54b	1000	1000	40	10	250	29.9	0.171	24.0
54c	1000	1000	40	10	250	38.0	0.384	33.3
55a	1000	1000	60	10	250	28.2	0.170	28.3
55b	1000	1000	60	10	250	63.6	0.299	26.2
55c	1000	1000	60	10	250	30.9	0.240	31.1
56a	1000	1000	80	10	250	64.0	0.347	28.8
56b	1000	1000	80	10	250	29.3	0.226	31.7
56c	1000	1000	80	10	250	64.6	0.500	33.8
57a	1000	1000	120	10	250	43.8	0.277	31.7
57b	1000	1000	120	10	250	30.0	0.196	30.9
57c	1000	1000	120	10	250	32.4	0.217	36.4

Table A6 Select Emulsions included in the phase diagram

Form.	Soy. Oil (mg)	T. 80 (mg)	P.90(mg)	Water	Z. Ave (nm)	Z.P(mV)	P.I
32a	1000	1000	250	10000	32.9	30.4	0.316
32b	1000	1000	250	10000	35.5	29.7	0.412
36a	1000	1000	500	10000	65	32.7	0.418
37a	1000	1000	100	10000	44.1	35.9	0.34
37b	1000	1000	100	10000	44.6	31.8	0.357
44a	1000	1000	250	20000	53.3	26.2	0.335
44b	1000	1000	250	20000	57.5	30.8	0.37
47a	1000	1000	100	20000	66.2	31.3	0.338
48a	1000	1000	100	15000	49.4	31.8	0.319
49a	1000	1000	250	15000	65	27.6	0.494
49b	1000	1000	250	15000	50.7	26.3	0.331
50a	1000	1000	500	15000	67.2	27.3	0.417
50b	1000	1000	500	15000	63.9	29.6	0.421

Table A7 Effect of Phospholipid concentration on Emulsion viscosity

Formulation	Surf./oil ratio	T. 80 (w/w%)	P. 90 (w/w%)	Viscosity
41a	0.36	7.07	0.71	0.8795
41b	0.36	7.07	0.71	0.8795
34a	0.41	6.99	1.74	0.8825
34b	0.41	6.99	1.74	0.8825
42a	0.49	6.87	3.40	0.8826
42b	0.49	6.87	3.40	0.8826
43a	0.58	6.76	5.07	0.8995
43b	0.58	6.76	5.07	0.8995

Table A9 Analysis of Variance HPLC

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Low	3	302.62	100.8733	15.11323
Medium	3	300.94	100.3133	2.420133
High	3	299.88	99.96	0.0976
Day 1	3	299.03	99.67667	2.777433
Day 2	3	300.28	100.0933	0.838933
Day 3	3	304.13	101.3767	12.29563

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Levels	1.272622	2	0.636311	0.08331	0.92162	6.944272
Days	4.710556	2	2.355278	0.308369	0.750671	6.944272
Error	30.55138	4	7.637844			
Total	36.53456	8				

Table A10. Drug Content Analysis Of Emulsions 3 and 17 by HPLC after 3 Months Stability Studies

40ºC	% Potency Day 0	% Potency 3 Months
Emulsion 3	113.5	62.62
Emulsion 17	106.81	36.04