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Design, Development, and Evaluation of Sunflower Oil Based Nanoemulsion of Fluconazole

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ABSTRACT

In my research study I have focused on the particle size reduction by sonication method as well as to increase the surface area of the particles that leads to increase the bioavailability of the formulation. Hence my aim was to design, formulate and optimized or evaluation of sunflower oil-based nano-emulsion of fluconazole for oral drug delivery. Fluconazole (bistriazole) is an antifungal agent with poor water solubility and lipophilicity. Sunflower oil is a natural, non-irritating, non-toxic that have a good tendency to permeation enhancer. Screening of emulsifiers like Tweens group includes Tween 20, tween 60, tween 80, and Spans group includes span 60, span 80 was done based on the solubility of fluconazole in these surfactants followed by their capability to emulsify the sunflower oil in water. In other hand Co-emulsifiers like such as glycols (polyethylene glycol 200, polyethylene glycol 400, propylene glycol), and short-chain alcohols (ethanol, propanol, and butanol) were also screened similarly. Then based on the solubility data Tween 80 and butanol were selected as emulsifier and co-emulsifier respectively for the formulation of nano emulsion by Aqueous titration method. But in this formulation separation was observed after 20 hours of storage condition. So, span 80 was also added as a secondary emulsifier to improve emulsification efficiency as well as to prevent the solution from separation. Finally, a blend of tween 80, span 80 and butanol was added as an emulsifier to emulsify sunflower oil in the ratio of 1:1:1. Then the formulation was optimized based on the data that includes characterization of viscosity, refractive index, pH, conductivity, dilution, drug content etc. Finally, the prepared formulation concluded that the developed sunflower oil-based nano-emulsion of fluconazole has effective solubility, permeability, bioavailability, and stability.

1. INTRODUCTION

1.1. Emulsions:

1.1.1. Description [1,26]

The emulsion is a biphasic liquid preparation having two immiscible liquid, one of which is dispersed as minute globules into another. The liquid which is converted into minute globule is called a dispersed phase/discontinuous/internal phase and the liquid in which the globules are dispersed is called a continuous phase/dispersion/External phase.

The size of globules should be 0.1 to 100 µm. This can be occurred due to the increase in surface tension between immiscible liquid and reduction in the particle size. And this can be possible by addition of a third substance which is called as Emulgent or Emulsifying agent. By adding the emulsifying agent, it forms a film around the globules in the continuous phase. Hence a stable emulsion is also formed.

1.1.2. Advantages [1,26]

- To mask the unpleasant taste:
- It is economical.
- Improve bioavailability.
- Sustained-release medication.
- As a nutritional supplement.
- Diagnostic purpose.
- Topical use.

1.1.3. Disadvantages [1,26]

- They are unstable the insoluble phase separates solely and is bulkiness in nature.
- Care should be taken in handling and storage of the formulation as it is liquid in nature and sometime comes in glass container.
- It has a shorter shelf life.

1.1.4. Classification [26]

A. Based on the nature of the dispersed phase:

- Oil in a water type (o/w)
- Water in oil type (w/o)

B. Based on the globule size:

- Microemulsion (0.01 μm)
- Nanoemulsion (Fine emulsion) (0.1 –0.5 μm)

C. Multiple Emulsion [42]

- W/O/W
- O/W/O

D. Based on their mode of administration [26]

- Emulsion for oral administration.
- Emulsion for external use.
- Emulsion for parenteral use.
- Emulsion for rectal use.

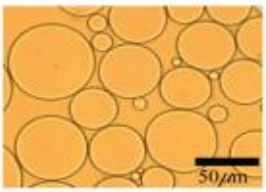
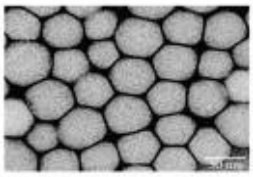
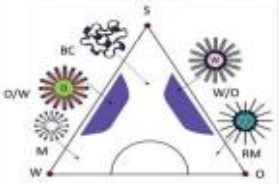
1.2. Nanoemulsion:

1.2.1. Description [18]

Nano emulsion is an emulsion of oil in water type with mean droplet diameters ranging from 50 to 1000 nm. but the average droplet size is between 50 and 500 nm. In this formulation the particles can exist either as oil-in-water or water-in-oil forms, where the core of the particle is either oil or water respectively. The surfactants used in this should be approved for human consumption and common food substances i.e. "Generally Recognized as Safe" (GRAS) by the FDA. The emulsifier sometimes may cause a critical problem for the formation of the smaller size particles droplets, because it decreases the interfacial tension that means the surface energy per unit area, between the oil and water phases of the emulsion. The emulsifier also helps in the stabilization of the

nanoemulsion through repulsive electrostatic interactions and steric hindrance. Generally, emulsifiers are used but we can also use proteins and lipids for the preparation of nano emulsions. The method use for formulation is broadly classified as high-energy and low-energy methods. The high energy methods that include ultrasonication as well as high pressure homogenization (HPH) to create about the small droplets size and the low energy methods that extend specific system properties to make small droplets size. Here are two examples of low energy approaches for the formation of nano emulsion that are Phase inversion temperature (PIT) and emulsion inversion point (EIP).

The range of nano emulsion applications extends to the fields of drug delivery, where O/W nanoemulsions have been used to deliver hydrophobic drugs i.e. water-hating drugs. In other hand the food industry, they are using the flavored nanoemulsions with improved curcumin / b-carotene and digestibility where that has been prepared, In the area of cosmeceutical industry where they are using it for skin hydration and for make easy of application of the cosmetic products. Apart from this there is some confusion in the literature regarding the definition of nanoemulsions, which are often confused about the thermodynamically stability of the microemulsions which form spontaneously. The major difference between emulsions (or macroemulsions), nanoemulsions and microemulsions are in the particle size and stability characteristics, as mentioned in below Fig. 1. Both the Macroemulsions and nanoemulsions are thermodynamically unstable, i.e. given sufficient time, phase separation occurs. [53] Therefore, because of the small size of nanoemulsions (sometimes also called ‘mini emulsions'), and they can be kinetically stable over long periods.

	macroemulsions	nanoemulsions	microemulsions
			
size	1-100 µm	20-500 nm	10-100 nm
shape	spherical	spherical	spherical, lamellar
stability	thermodynamically unstable, weakly kinetically stable	thermodynamically unstable, kinetically stable	thermodynamically stable
method of preparation	high & low energy methods	high & low energy methods	low energy method
polydispersity	often high (>40%)	typically low (<10-20%)	typically low (<10%)

(Fig. 1 Comparation in between Macro emulsion, Nano emulsion and Micro emulsion) [51]

1.2.2. Types of Nano emulsions [33]

Based on components used, they are classified as

1. Oil in water nano emulsions: Here oil droplets are dispersed in the continuous aqueous phase.

2. Water in oil nano emulsions: Here water droplets are dispersed in the continuous oil phase.

3. Bicontinuous nano emulsions: Here microdomains of oil and water are inter dispersed within the system.

1.2.3. Advantages of Nano emulsions as drug delivery systems [30,31]

1. These are thermodynamically and kinetically stable thus preventing flocculation, aggregation, creaming, and coalescence.

2. It is a mostly use approach to improve water solubility and bioavailability of lipophilic drugs.

3. It can be administered by various routes like such as oral, topical, parenteral and transdermal, etc.

4. It can deliver both hydrophilic and lipophilic drugs.

5. It has the potential to deliver peptides that are prone to enzymatic hydrolysis in GIT.

6. It is nano size droplet, so surface area is large thus increases the rate of absorption and decreases variability, thus enhances the bioavailability of drugs.

7. Suitable for human and veterinary use because It does not damage or harm to the human or animal cell.

8. It also protects the drug from hydrolysis and oxidation by encapsulating in the oil-droplet. And also provides taste masking.

9. It also enhances the permeation of drug through the skin.

10. It also provides ultra-low interfacial tension and a large o/w interfacial area.

1.2.4. Disadvantages of nanoemulsion drug delivery systems [32]

1. A large concentration of surfactants /cosurfactants is required for stabilization.

2. Its stability effect by temperature and pH.

3. Oswald ripening effect also causes instability of the formulation.

4. It is an expensive process, because of size reduction.

1.2.5. Applications of Nanoemulsion [34,35]

It contains active pharmaceutical ingredients (API) that can be utilized for the invention of pharmaceutical formulations. It also contains a suitable vehicle for therapeutic administration of the drugs. In some case a special galenic form can be imparted to the mixture. Some of the galenic forms of administration are ampoules especially for sterile injection and infusion solutions, solutions especially for oral liquids, eye drops and nose drops which can contain various secondary substances in addition to the nanoemulsion and in aerosols without metering feature and dosing the aerosol contain propellant gas and stabilizers. Besides the nanoemulsion certain hydrophilic and hydrophobic gels and ointments also contains nanoparticles.

1. Ocular Delivery: The O/w emulsion are being used for the improvement of topical lipophilic drug delivery to the eye. Lipophilic drug loaded o/w ocular emulsions can provide a better balance in between the ocular bioavailability response and patient comfortness. E.g. pilocarpine, indomethacin, piroxicam cyclosporine A.

2. Nasal Route: This route has been a great attention now a days because of its several advantages over parenteral and oral administration especially bypass to the liver. It increases the absorption by solubilizing the drug in the inner phase of an emulsion and increases the contact time between emulsion droplets and nasal mucosa. Drugs like insulin and testosterone are being formulated for nasal drug delivery.

3. Use of Nanoemulsions in Cosmeceuticals: Nowadays the nanoemulsion has been an important potential vehicle for the controlled delivery of cosmetics and in the optimization dispersion of active ingredients in particular skin layers. Nanoemulsions are more suitable for the transport of lipophilic compounds than liposomes because of its lipophilic nature. As like liposomes it also supports the skin penetration of active pharmaceutical ingredients and thus increases their concentration in the skin. One of the main advantages of the nanoemulsion is formation of small-size particles droplet with its high surface area that allow for effective transport of the action protentional to the skin. It is also in demand because of its bioactive effects. It can also reduce the trans epidermal water loss, and that indicates that the barrier function of the skin is strengthened. One of the more advantages is that there is no creaming, sedimentation, flocculation or coalescence observed and That's why it has been in demand in cosmeceutical.

4. Antimicrobial Nanoemulsions: It is one of the emulsions i.e. oil in water droplets having a size range from 200-600 nm. Here the particles are thermodynamically fused with lipid-containing organisms. When sufficient nanoparticles fuse with the pathogens, they release part of the energy trapped within the emulsion. Both of the active ingredient as well as the energy released destabilize the pathogen lipid membrane, resulting in cell lysis and death. It has broad-spectrum activity against bacteria (e.g. E. Coli, Salmonella, S. aureus) enveloped viruses (e.g. HIV, Herpes Simplex), Fungi (e.g. Candida, Dermatophytes) and spores (e.g. anthrax).

2. MATERIALS & METHODOLOGY

2.1. Materials:

2.1.1. Chemical and reagents:

Fluconazole was received from Dr. Reddy's Laboratory, Hyderabad, Tween 20, Tween 60 and Tween 80 were purchased from National chemicals (Baroda), Span 60 and Span 80 were purchased from Loba Chemie (Pvt.) Ltd., Mumbai, India, Polyethylene glycols, propylene glycol, isopropyl alcohol, n-butanol, ethanol were purchased from Suvidhinath laboratories (Baroda) and sunflower oil was purchased from the local market, water was used for all the experiments throughout the study.

2.1.2. Excipients profile:

2.1.2.1. Fluconazole:[44]

Chemical Name: 2-(2,4-difluorophenyl)-1,3-Bis(1H-1,2,4-triazol-1-yl) propan-2-ol

Chemical formula: $C_{13}H_{12}F_2N_6O$

Molecular weight: 306.277 g/mole

Description: Fluconazole is a broad-spectrum antifungal agent, active by both oral and intravenous routes, for the treatment of superficial and systemic infections. It is a triazole compound and has established exceptional therapeutics record for candida infections including oropharyngeal and esophageal candidiasis, vulvovaginal candidiasis, candidemia, and disseminated candidiasis. It presents a reasonable solubility and is more hydrophilic than other azole derivatives used as antifungals where this leads to better absorption from the gastrointestinal tract. Although fungi and human cells are eukaryotic and are similar at a biological level, this drug perfectly differentiates between these two.

Stability and Storage Conditions: Fluconazole is stable for at least 1 year when stored at a cool temperature (< 30^0C) and less than 50% relative humidity. Its solutions may require the addition of surfactants and cosurfactants. It should be stored in a well-closed container in a cool, dry place.

2.1.2.2. Span 60: [41]

It is also called as Sorbitan monostearate. It is an ester of sorbitan (a sorbitol derivative) and stearic acid and it is sometimes referred to as a synthetic wax. It is very soluble in water. The stearic acid end is soluble in fats. Hence it is very good at making emulsions of oil and water. It is used in the manufacture of food and healthcare products and is a non-ionic surfactant with emulsifying, dispersing, and wetting properties.

IUPAC Name: [2-[(2R,3S,4R)-3,4-dihydroxyoxolan-2-yl]-2-hydroxyethyl] octadecanoate.

Molecular Formula: $C_{24}H_{46}O_6$

Molecular Weight: 430.62 (g/mol)

Synonym - Span 60, emulsifier S60

Description - Generally It is white to tan solid wax, soluble in hot ethanol, benzene, and hot oil; slightly soluble in ether and petroleum ether and insoluble in propylene glycol [143]. Mainly it is a water/oil type emulsifier. The HLB value is 4.7. Its odor is about slight and taste about bland. The melting point of span 60 is about 57°C.

Absorption, distribution, and excretion: When digested, both the fatty acid and the polyhydric alcohol sorbitan are absorbed, but the latter is completely excreted in the urine.

Uses: It is used as an anti-static agent, emulsifier and stabilizer in medicine, cosmetics, food, pesticide, coatings, plastic and textiles industries.

Stability and Storage Conditions: Gradual soap formation occurs with strong acids or bases; sorbitan esters are stable in weak acids or bases. Span 60 should be stored in a well-closed container in a cool, dry place.

2.1.2.3. Tween 80: [41]

It is also called as Polysorbate 80. It is an ester of sorbitan (a sorbitol derivative) and stearic acid and is sometimes referred to as a synthetic wax. It is also very soluble in water. The stearic acid end

is soluble in fats. Hence it is very good at making emulsions of oil and water. It is used in the manufacture of food and healthcare products and is a non-ionic surfactant with emulsifying, dispersing, and wetting properties.

Molecular Formula: $C_{64}H124O_{26}$

Molecular Weight: 1310 (g/mol)

Synonym – Tween 80

Description: It has the characteristic odor and a warm, bitter taste. Its color at 258C is yellow oily liquid, although it should be noted that the absolute color intensity of the products may vary from batch to batch and from manufacturer to manufacturer. Generally, it is soluble in water, hot ethanol, benzene, and hot oil; and slightly soluble in ether and petroleum ether and insoluble in propylene glycol. It is mainly a water/oil type emulsifier. HLB value: 15.0, melting point of Tween 80 is about 57°C.

Absorption, distribution, and excretion: When digested, both the fatty acid and the polyhydric alcohol sorbitan are absorbed, but the latter is completely excreted in the urine.

Used: It is used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions. They are useful in improving the oral bioavailability of drug molecules that are substrates for p-glycoprotein. It is also used for cosmetics and food products.

Stability and Storage Conditions: Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. It is hygroscopic and should be examined for water content before use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides. It should be stored in a well-closed container and protected from light, in a cool, dry place.

2.1.2.4. Sunflower oil: [41]

It is a viscous liquid obtained from the fruits and seeds (achenes) of the sunflower, Helianthus annus (Compositae), by mechanical means or by extraction. It also called as Huile de tournesol; oleum helianthi; sunflower seed oil. It is classified as an oleic–linoleic acid oil. It is composed of

linoleic acid (66%), oleic acid (21.3%), palmitic acid (6.4%), arachidic acid (4.0%), stearic acid (1.3%), and behenic acid (0.8%). On Functional Category: Diluent; emollient; emulsifying agent; solvent; tablet binder

Uses: In Pharmaceutical Formulation or Technology it is widely used as an edible oil, primarily in oleomargarine. It is also used extensively in the cosmetics and in the pharmaceutical formulations. Therapeutically, it is also used to provide energy and essential fatty acids for parenteral nutrition. In previous studies it has shown that sunflower oil may be used in intramuscular injections without inducing tissue damage.

Description: Sunflower oil occurs as a clear, light yellow-colored liquid with a bland, agreeable taste. Typically, Boiling point: 40–608C, Density: 0.915–0.919, Hydroxyl value: 14–16, Iodine number: 125–140, Melting point: 188C, Refractive index: 1.472–1.474, Saponification number: 188–194. Its solubility is miscible with benzene, chloroform, carbon tetrachloride, diethyl ether, and light petroleum; practically insoluble in ethanol (95%) and water.

Stability and Storage Conditions: Sunflower oil should be stored in an airtight, well-filled container, protected from light. Stability may be improved by the addition of an antioxidant such as butylated hydroxytoluene.

Incompatibilities: Its stability is reduced in the presence of iron oxides and zinc oxide and it forms a ‘skin’ after being exposed to air for 2–3 weeks.

Uses: Food products as edible oil, cosmetics, and topical pharmaceutical formulations, Generally regarded as a relatively nontoxic and nonirritant material.

2.1.2.5. Span 80: [41]

It is also called as Sorbitan monooleate is an ester of sorbitan (a sorbitol derivative) and stearic acid and is sometimes referred to as a synthetic wax. It is very soluble in water and the stearic acid end is soluble in fats that’s why it is mostly used for making emulsions of oil and water. Generally, it is used in the manufacture of food and healthcare products and is a non-ionic surfactant with emulsifying, dispersing, and wetting properties. IUPAC Name is [2-[(2R,3S,4R)-3,4-dihydroxyoxolan-2-yl]-2-hydroxyethyl] octadecanoate.

Molecular Formula: $C_{18}H_{34}O_6$

Molecular Weight: 346 (g/mol)

Synonym - Span 80, emulsifier S80

Description – Span 80 is yellow viscous liquid. It is soluble in hot ethanol, benzene, and hot oil; slightly soluble in ether and petroleum ether and insoluble in propylene glycol. Span 80 is water/oil type emulsifier. HLB value: 4.3. Its odor is about slight and taste about bland. The melting point of span 80 is about 57°C.

Absorption, distribution, and excretion: When digested, both the fatty acid and the polyhydric alcohol sorbitan are absorbed, but the latter is completely excreted in the urine.

Uses: Generally, it is used as an anti-static agent, emulsifier and stabilizer in medicine, cosmetics, food, pesticide, coatings, plastic and textiles industries.

Stability and Storage Conditions: Gradual soap formation occurs with strong acids or bases; sorbitan esters are stable in weak acids or bases. Span 60 should be stored in a well-closed container in a cool, dry place.

2.2. Methods of Nano emulsion Preparation: [4]

The Nano emulsion can be prepared by different methods that includes high energy method or low energy methods. In high energy method require large energy force for mechanical device deliver, but in low energy method there is no external force is required. In the time of initial studies of nano emulsion, the high energy method was the only choice for the researcher and thus high energy stirring and ultrasonic emulsification were the most widely used methods (Korelova & Yurtov,2012). But in this time the low energy method has drawn a considerable attention because of the softness, non-destructive and it does not damage to encapsulated molecules (Anton et al,2008).

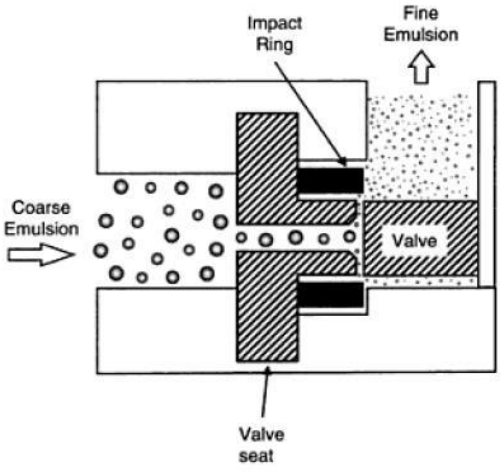
2.2.1. High Energy Emulsification methods:

Nano emulsion is a non-equilibrium system which cannot be formed spontaneously. That's why a mechanical or chemical energy input s necessary. It is generally prepared by using high energy methods in which mechanical energy input is applied by high pressure homogenizer, high shear stirring and ultra sound generators (Sole et al,2012). This mechanical device provides strong forces that disrupt oil and water phases to form nano emulsion. In high energy method, the input energy density is about 108-1010 W Kg-1 (Gupta et al,2016). The requires energy is supplied in a shorter time period to the system to obtain homogeneous small size particles. The High-pressure

homogenizers are capable of doing this and therefore it is the most wisely used device for the preparation of nano emulsion (Sloan's et al,2005).

2.2.2. High-Pressure Homogenization Methods:

It is the most popular method used for the production of nano emulsions. This method benefits from the high-pressure homogenizer or the piston homogenizer (Figure no 2) to manufacture nano emulsions that particle sizes are up to 1 nm. During the method, the macroemulsion is forced to pass through in a small orifice at an operating pressure between 500 to 5000 psi (Chime et al., 2014). Extremely small droplet sized nano emulsions are achieved because during the process several forces like hydraulic shear, intense turbulence and cavitation act together.



(Fig No.2: Schematic Representation of High pressure valve homogenizer)

(Mc Clements et al,2005)

This process can be repeated until the final product reaches the desired droplet size and polydispersity index (PDI). The uniformity of droplet size in nanoemulsions is specified by PDI (Jaiswal et al., 2015). Higher PDI means lower uniformity of droplet size in nanoemulsions. Monodisperse samples have PDI lower than 0.08, PDI between 0.08 and 0.3 states a narrow size distribution, whereas PDI greater than 0.3 indicates broad size distribution (Zhang, 2011). However, obtaining of small droplets that are in submicron levels requires a large amount of energy (Lovelyn & Attama, 2010). This amount of energy and increasing temperatures during high-pressure homogenization process might cause deterioration of the components (Setya et al., 2014). Thermolabile compounds such as proteins, enzymes, and nucleic acids may be damaged (Floury et al., 2000; Chime et al., 2014).

2.2.3. High-Shear Stirring Methods:

In this method, high-energy mixers and rotor-stator systems are used for the preparation of nanoemulsions. Droplet sizes of the internal phase can be significantly decreased by increasing the mixing intensity of these devices. However, obtaining emulsions with the average droplet size of less than 200-300 nm is rather difficult (Korelova & Yurtov, 2012).

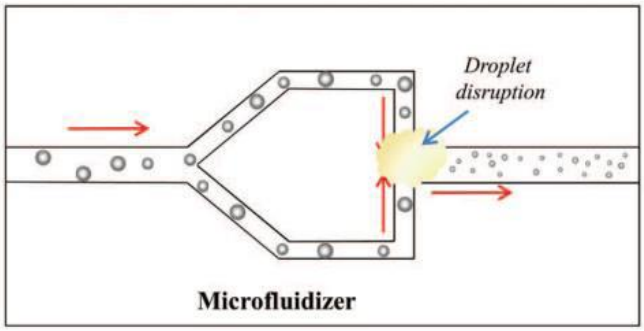
2.2.4. Ultrasonic Emulsification Methods:

There are two mechanisms which take part in ultrasonic emulsification. Firstly, the acoustic field creates interfacial waves that make the oil phase to disperse in the continuous phase as droplets. Secondly, ultrasound provokes acoustic cavitation which provides formation and collapse of microbubbles respectively due to pressure fluctuations of a single sound wave. In this way, enormous levels of highly localized turbulence are generated and this causes micro implosions which disrupt large droplets into sub-micron size (Zhang, 2011). In this method, premixed macroemulsion is agitated by vibrating solid surface at 29 kHz or larger frequencies. High-power ultrasonic devices such as focusing horns and pointed tips cause extreme shear and cavitation that result in breaking up of droplets. It has been observed that in most of the ultrasonic systems emitted sound field is inhomogeneous. For this reason, to have all droplets to experience the highest shear rate, recirculation of the emulsion through the region of high power must be provided. Moreover, by doing this type of recirculation many times it is possible to obtain emulsions with uniform droplet size at dilute concentrations (Mason et al., 2006). Emulsifier type, the amount emulsifier, and viscosity of phases are the most critical parameters that affect homogenization efficiency (Maa & Tsu, 1999; Leong et al., 2009). Thus, optimization of these parameters is necessary to prepare nano emulsions having fine droplets. However, there are some concerns about sonication methods because they can induce protein denaturation, polysaccharide depolymerization and lipid oxidation (Jafari et al., 2006; McClements & Rao, 2011).

2.2.5. Microfluidization Methods:

It is most widely employed in the pharmaceutical industry to acquire fine emulsions. In this method, a device called microfluidizer is used which provides high pressures (Figure 3). During the process, high pressure forces the macroemulsion to go through to the interaction chamber and thus nanoemulsions with submicron ranged particles can be produced. Uniform nanoemulsion production can be achieved by repeating the process many times and varying the operating pressure to get the desired particle size (Chime et al., 2014; Jaiswal et al., 2015). There is a collision between

crude emulsion jets from two opposite channels in the nozzle of microfluidizer which is also called as the interaction chamber. The mobility of crude emulsion is provided by a pneumatically powered pump that has the capability of compressing air up to pressures between 150 to 650 MPa. This high pressure forces the crude emulsion stream to go through microchannels and after the collision of two opposite channels enormous level of shearing force is obtained. Therefore, by the help of this force fine emulsions are produced (Gupta et al., 2010).



(Fig No.3.Schematic representation of microfluidizer (McClements & Rao et al,2011).

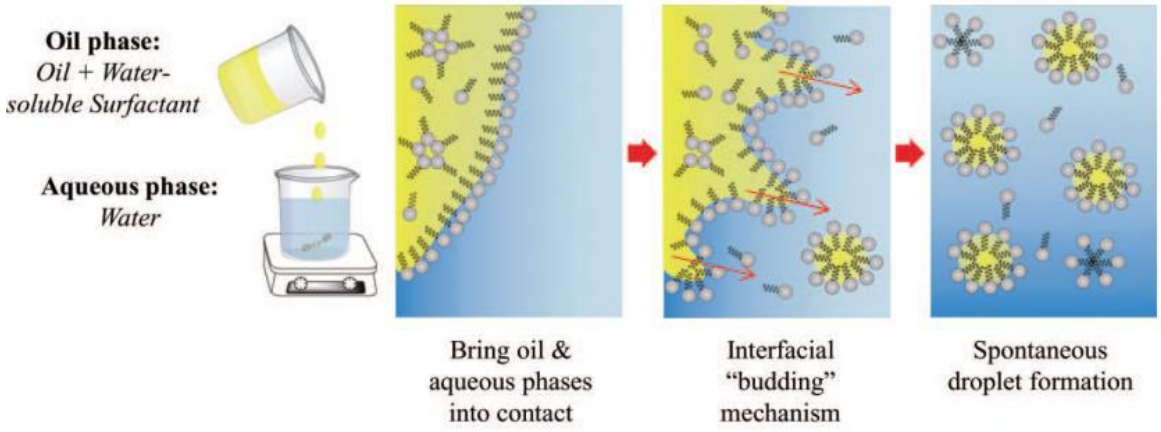
2.2.6. Low-Energy Emulsification Methods:

It can also be achieved with low-energy methods which provide small size and more uniform droplets (Solans et al., 2005; Sole et al., 2012). These methods such as phase inversion temperature and phase inversion component provide smaller and more uniform droplets by using physicochemical properties of the system (Caldero et al., 2011). Although low energy procedures are generally more effective to produce small droplet sizes than high energy procedures, there are some limitations for them about the using of some types of oils and emulsifiers like proteins and polysaccharides. To overcome this problem high level of synthetic surfactant concentrations are used to produce nano emulsions in low energy techniques but this narrows down their application area, especially for many food processes (McClements & Rao, 2011).

2.2.7. Spontaneous Nano emulsification Methods:

It benefits from the chemical energy releasement based upon dilution process with the continuous phase which occurs usually at a constant temperature without any phase transitions in the system during the emulsification process (Sloan's & Sole, 2012). This method can produce nano emulsions at room temperatures and no special devices are required. It subjected to interfacial tension, the viscosity of interfacial and bulk, phase transition region, surfactant structure, and surfactant concentration (Setya et al., 2014). In the pharmaceutical industry, systems prepared by using this

method are usually called as self-emulsifying drug-delivery systems (SEDDS) or self-nano-emulsifying drug-delivery systems (SNEDDS). When an oil phase with a water-soluble substance is mixed with water, oil droplets spontaneously forms. The mechanism depends on the movement of a water-dispersible substance from the oil phase to the water phase, indicated as red arrows in Figure. 4. This leads to interfacial turbulence and thus the formation of spontaneous oil droplets (McClements & Rao, 2011).



(Fig no.4.Schematic representation for spontaneous emulsification (McClements & Rao et al, 2011)

2.2.8. Phase Inversion Methods:

These methods utilize the chemical energy that is released because of the phase transitions during the emulsification process (Anandharamakrishnan, 2014). The required amount of phase transitions is achieved by changing the composition at a constant temperature or by changing the temperature at constant composition (Thakur et al., 2013).

2.2.9. Phase Inversion Temperature (PIT) Methods:

In this method, the temperature is changed at constant composition. Non-ionic surfactants which have temperature-dependent solubility like polyethoxylated surfactants play an important role. Emulsification is achieved by modifying the affinities of surfactants for water and oil as a function of temperature (Lovelyn & Attama, 2010; Chime et al., 2014). During heating of polyethoxylated surfactants, they become lipophilic due to dehydration of polyoxymethylene groups. Therefore, this circumstance establishes the principle of producing nanoemulsions by PIT method. To prepare nanoemulsions by using the PIT method, it is necessary to bring sample temperature to its PIT level or hydrophile–lipophile balance (HLB) level (Anandharamakrishnan, 2014). In the PIT method, the droplet sizes and the interfacial tensions reach their minimum value. This method promotes

emulsification by benefiting from the extremely low interfacial tensions at the HLB temperature. Nevertheless, it has been observed that although emulsification is spontaneous at the HLB temperature, the coalescence rate is greatly fast and emulsions are highly unstable (Ee et al., 2008). It has been reported that stable and fine emulsion droplets can be produced by the rapid cooling of the emulsion near the temperature of PIT (Tadros et al., 2004; Rajalakshmi et al., 2011).

2.2.10. Phase Inversion Composition (PIC) Methods:

In this method, the composition is changed at a constant temperature. Nanoemulsions are obtained by consistently adding water or oil to the mixture of oil-surfactant or water-surfactant. The PIC method is more suitable for a large-scale production than the PIT method since adding one component to an emulsion is easier than to generate abrupt change in temperature (Solans & Sole, 2012). By adding water to the system, the volume of water increases and this result to reach a transition composition. In other words, the level of hydration of the polyoxyethylene chains of the surfactant increases and thus spontaneous curvature of the surfactant goes to a change from negative to zero. As in the HLB temperature, in the transition composition, a balance is obtained for the surfactant hydrophilic-lipophilic properties. When this transition composition is exceeded, small-sized metastable oil in water droplet is composed due to the separation of the structures that have zero curvature (Anandharamakrishnan, 2014).

2.3. Methodology:

2.3.1. Preformulation study:

It is the first step in the rational formulation of an active pharmaceutical ingredient (API). It is an investigational study about the physic-chemical properties of the drug substance and in combination with excipients. The studies about the active drugs/excipient compatibility represent an important phase in the preformulation stage of the development of all dosage forms. The physical and chemical interactions between drugs and excipients can affect the chemical nature, the stability, and bioavailability of drugs and, consequently their therapeutics efficacy and safety. Drug with an individual excipient, all the excipients together were kept in closed glass containers at 40^0C and 75% relative humidity (RH) for 6 months to inspect any physical changes under the condition described.

a. Drug – Excipients compatibility study

b. Excipient – Excipient compatibility study.

2.3.2. Screening of Oils, Surfactants, and Cosurfactants for Nanoemulsion formation: [44]

The most important criteria for the screening of oil for nanoemulsion is the maximum solubility and compatibility with the drug. Based on the biocompatibility profile of oils from the literature review, the drug solubility was determined. Excess amount of drug ie 100mg in 3mL of selected Sunflower oils was taken in stopper vials and was then mixed by vortex mixer. The mixture vials were then kept at 37±2.0 o C in an isothermal orbital shaker for 72 hr to reach equilibrium. Then the equilibrated samples were removed from the shaker and centrifuged at 5000 rpm for 15 min. Then the solubility profile of the drug in oil was determined from the supernatant using UV-VIS spectrophotometer at 257 nm. Then the insoluble drug from the settled material was determined and the mass balance was found out.

2.3.3. Solubility studies of Fluconazole:

The solubility studies were performed by phosphate buffer saline (PBS) pH 7.4, butanol, Ethanol, Sunflower oil as well as water in the ratio of 9:1 by adding excess amounts of the drug in each case and keeping the excess amount of drug-containing phosphate buffer flasks on a rotary shaker for 24 h. After 24 h, solutions were analyzed using UV Visible spectrometry against PBS pH 7.4 as a blank and drug concentration was calculated.

2.3.4. Emulsion efficiency of Emix and preparation of Emix:

Based on solubility data, tween 80 and propylene glycol were chosen as emulsifier and co-emulsifier respectively. An equal part of tween 80 and propylene glycol was mixed with varying amounts of sunflower oil to prepare different ratio of oil: Emix. These mixtures were then titrated with water and observations are recorded. Aqueous titration of oil: tween 80: propylene glycol, resulted in the formation of either emulsion or gel. Therefore, emulsification efficiency of different co-emulsifiers was tested at 1:1 ratio of Emix (co-emulsifier: emulsifier, taking tween 80 as an emulsifier in each case). Emulsification efficiency of these Emix was evaluated based on the formation of nano-emulsions with a minimum amount of Emix consumed in the titration of equal parts of water and sunflower oil. Butanol was nanoemulsion, however, there was some sign of separation after 24 hours. Therefore, different emulsifiers were further tested for emulsification efficiency keeping butanol as co-emulsifier in each case. Emulsion efficiency data has been presented in Table. It was observed that none of the emulsifiers alone was capable of forming stable nano-emulsion while the emulsifying equal volume of sunflower oil and water tested as several strengths up to 80% v/v of the total mixture. Therefore, span 80 was tested as an auxiliary

emulsifier with tween 80. Finally, Emix containing tween 80, span 80 and butanol at 1:1:1 ratio was found to successfully emulsify olive oil and water without application of any force or energy upon simple vortexing for 30 seconds.

2.3.5. Preparation of Nano emulsion:

The sunflower oil-based nanoemulsion of fluconazole was prepared by Self-Nanoemulsification Method (Aqueous titration method). In this method the generation of nanoemulsions at room temperature without the use of any organic solvent and heat. The stable nanoemulsions kinetically with small droplet size (~50 nm) can be generated by the stepwise addition of solution (Emix) of surfactant in oil with butanol into the water, with gentle stirring and at a constant temperature. The spontaneous nano emulsification has been related to the phase transitions during the emulsification process and involves lamellar liquid crystalline phases or D-type bicontinuous microemulsion during the process. Nanoemulsions obtained from the spontaneous nano emulsification process ie by sonication of the solution. These are not thermodynamically stable, although they might have high kinetic energy and long-term colloidal stability.

2.4. CHARACTERIZATION ASPECTS:

2.4.1. Viscosity:[45] The viscosity was measured to determine the rheological properties of formulations. Brookfield Rheometer viscometer at 30°C with a CPE 61 spindle at 30 rpm was used to serve this purpose. Results were taken in triplicate and the average was taken into consideration.

2.4.2. PH Value: [46] The PH value of the formulation is an important parameter of the Nano emulsion formulation. Its value is dependent on the excipients that are used for the preparation of the formulation and also to the route where to be administered. The PH value of the formulation was measured by using digital PH meter. The result was taken in triplicate and the average value was taken into consideration.

2.4.3. Drug content:[46] The drug content of Drug nano emulsion formulation was measured using UV visible spectroscopic method. The 2 µg/ml of aliquot was prepared using nanoemulsion formulation using a diluting solvent. The samples were measured as 278.2 nm using UV-VIS spectroscopic method. Results were taken in triplicate and the average was taken into consideration.

2.4.4. Centrifugation:[47] This parameter was characterized to check physical stability. The nanoemulsion system was centrifuged at 5000 rpm for 10 minutes to determine whether the system shows signs of creaming or phase separation. The system was observed visually for appearance.

2.4.5. Conductivity: [47] Electrical conductivity of formulated samples was measured using a digital conductometer at ambient temperature. Results were taken in triplicate and the average was taken into consideration.

2.4.6. Dilution test: [48] If the continuous phase is added in nano emulsion, it will not crack or separate into phases. The maximum amount of water and oil were added to o/w and w/o formulations respectively and then inspected visually for clarity and phase separation. Here 50 times aqueous dilution of the formulation was visually checked for phase separation and clarity. Results were taken in triplicate and the average was taken into consideration.

2.4.7. Refractive Index:[48] The refractive index of the drug-loaded formulation was estimated on Rudolph Refractometer evaluated the refractive index of the optimized formulation in triplicate.

2.4.8. Stability of Drug nano emulsion: [49] Samples of Drug nano emulsion formulations was sealed in ampoules and then placed in Stability chambers at different temperature conditions i.e., room temperature (25^0C) and accelerated temperature (40 ± 2^0C) for 2 months. Duplicate samples were withdrawn at 0, 1 and 2 months to evaluate their physical and chemical stabilities. The physical stability was evaluated by visual inspection for physical changes (such as phase separation and drug precipitation), and a globule size analyzer was used to determine the mean globule size and zeta potential after dilution with water. Chemical stability was expressed as the content of Drug determined by UV visible spectroscopic method at 266 nm.

2.4.9. Light microscopy: Small amounts of the solution were taken and were spread on a glass slide, and then examined for the vesicle structure and the presence of insoluble drug crystals using ordinary light microscope with varied magnification powers (4x and 10x). Photomicrographs were taken using a digital camera (Sony Cybershot DSC-W690 16.1 megapixel). Each experiment was carried out in triplicate.

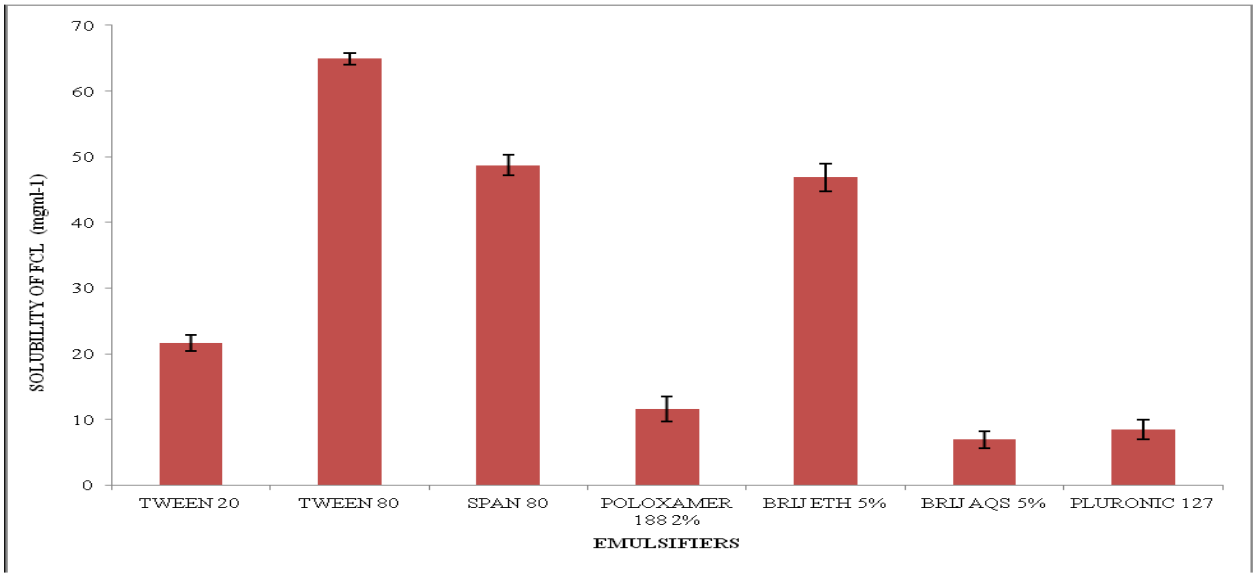
2.4.10. Fourier transforms infrared (FTIR) spectroscopy: The FTIR spectra of pure Fluconazole blank and optimized formulation (F2) were recorded using a Jasco FTIR spectrophotometer (Model FTIR-4100, Jasco, USA) according to the KBr pellet technique to examine any possible interaction between drug and excipients. The FTIR measurements were performed with a constant resolution of 0.9 cm^{-1} in the scanning range of 4000-500 cm^{-1} at ambient temperature.

3. Table & Figures:

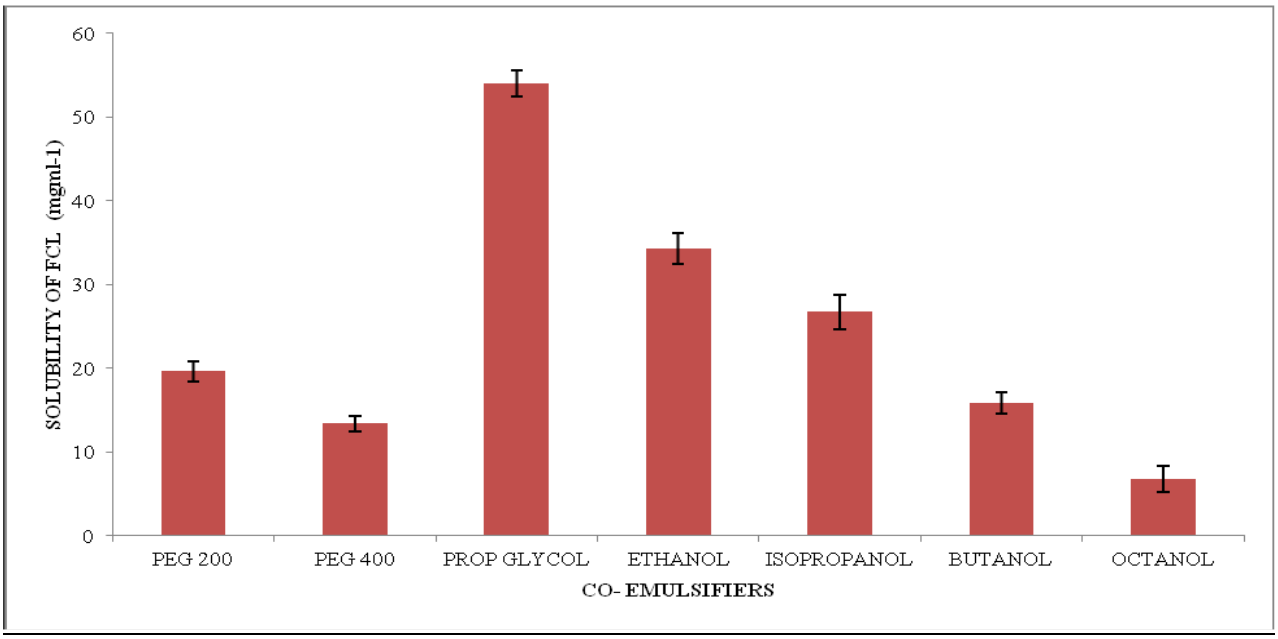
3.1. Table 1(Observation report of sunflower oil with Emix in Aqueous titration method)

For mulation	The ratio of oil: Emix		Observation after every 15 minutes with the addition of the solution.												
	Oil	Emix	*O min*	*15 min*	*30 min*	*45 min*	*60 min*	*75 min*	*90 min*	*105 min*	*120 min*	*135 min*	*150 min*	*165 min*	*180 min*
1	12 ml	59 ml	*E*	*E*	*E*	*E*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*
2	12 ml	75 ml	*E*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*	*NE*
3	10 ml	56 ml	*G*	*G*	*G*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*
4	10 ml	72 ml	*G*	*G*	*G*	*G*	*G*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*
5	7 ml	53 ml	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*N*	*N*	*N*	*N*
6	8 ml	60 ml	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*
7	9 ml	56 ml	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*N*	*N*	*N*
8	11 ml	59 ml	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*N*	*N*	*N*	*N*	*N*
9	13 ml	53 ml	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*N*	*N*	*N*	*N*
10	14 ml	54 ml	*G*	*G*	*G*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*	*E*

[E-Emulsion, NE-Nano emulsion, G-Gel]



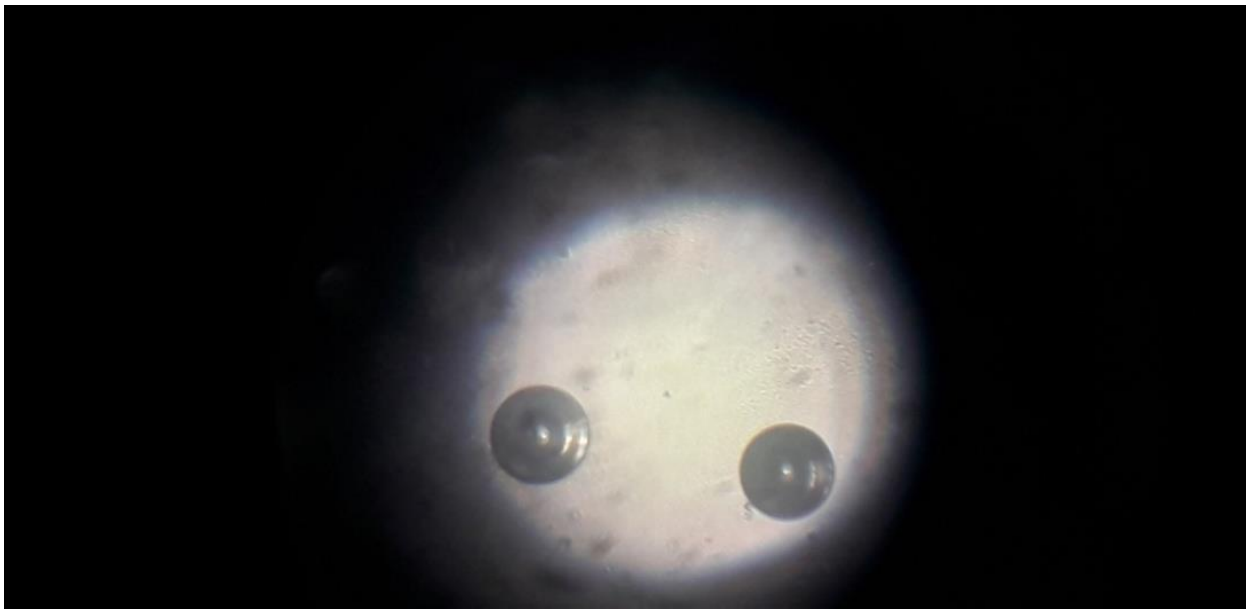
3.2. Fig No.5: Screening of co-emulsifier based on solubility data: [4]



3.3. Fig No.6: Screening of co-emulsifier based on solubility data: [4]

3.4.Table No.2: Effect of Emix with water and oil in different ratio:

Emix composition	Ratio	% v/v of Components of Nanoemulsions			Observation
		Emix	Sunflower oil	Water	
Tween 80: PEG 200	1:1	80	10	10	Gel
Tween 80: PEG 400	1:1	80	10	10	Gel
Tween 80: PEG	1:1	80	10	10	Gel
Tween 80: Ethanol	1:1	80	10	10	Emulsion
Tween 80: Butanol	1:1	80	10	10	Separate
Tween 80: Butanol	2:1	80	10	10	Separate
Tween 80: Butanol	3:1	80	10	10	Separate
Tween 80: Butanol	4:1	80	10	10	Separate
Tween 80: Butanol	1:2	80	10	10	Separate
Tween 20: Butanol	1:1	80	10	10	Separate
Tween 60: Butanol	1:1	80	10	10	Separate
Span 60: Butanol	1:1	80	10	10	Separate
Tween 80: Span 80: Butanol	1:1:1	76.2	11.9	11.9	Nanoemulsion

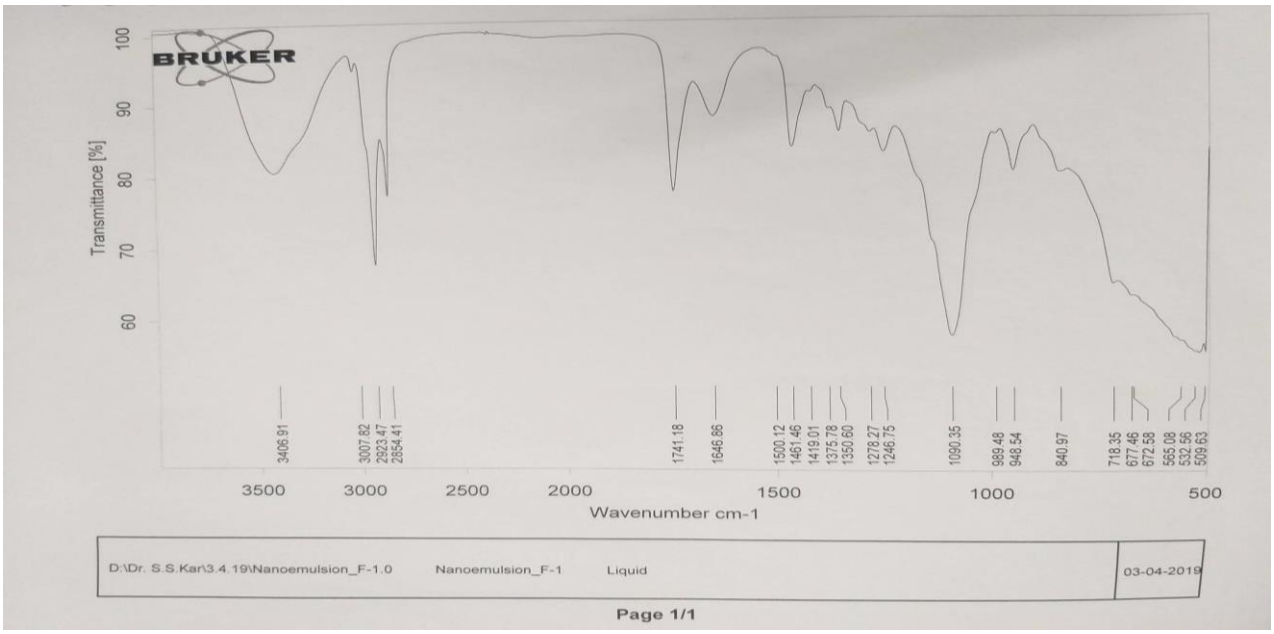


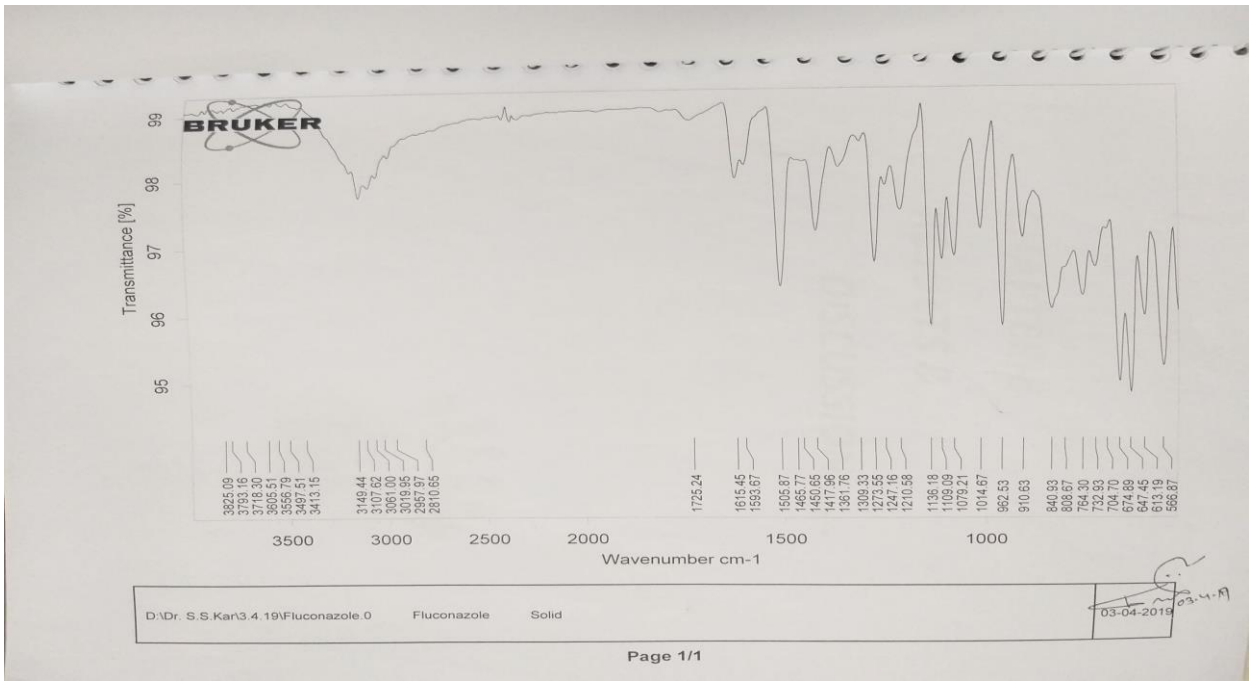
3.5. Figure No.7: Morphology of Nano emulsion droplets

3.6. Table No.3: Prepared Nano emulsion In different ratio:

Formulation	Mass(mg) of Drug, oil, emulsifier, emulsifier and water					
	Fluconazole (API)	Sunflower Oil	Tween 80	Span 80	Butanol	water
1	1 gm	12 ml	22 ml	20 ml	17 ml	28 ml
2	1 gm	12 ml	28 ml	24 ml	23 ml	12 ml
3	1 gm	10 ml	21 ml	19 ml	16 ml	33 ml
4	1 gm	10 ml	23 ml	21 ml	27 ml	18 ml
5	1 gm	8 ml	20 ml	18 ml	15 ml	38 ml
6	1 gm	8 ml	20 ml	21 ml	15 ml	35 ml
7	1 gm	10 ml	21 ml	19 ml	16 ml	33 ml
8	1 gm	12 ml	22 ml	20 ml	17 ml	28 ml
9	1 gm	13 ml	16 ml	13 ml	16 ml	32 ml
10	1 gm	14 ml	18 ml	19 ml	20 ml	28 ml

3.7. Figure No.8. FTIR Reports:





4. RESULTS & DISCUSSION:

4.1. Solubility study: From the solubility profiles, the drug concentration of Fluconazole was found to be 20 mg/ml in water, 50 mg/ml in oil, 148.6 mg/ml in Ethanol, 101.4 mg/ml in Butanol and 143.5 mg/ml at pH 1.2, 4.5 and 7.4 respectively.

4.2. Viscosity Estimation: The viscosity value was estimated to determine the rheological properties of the formulation. The formulation was measured by Brookfield Rheometer Viscometer at 30°C with a CPE 61 spindle at 30 RPM. Results were taken in triplicate and the average was taken in to consideration. The estimated result was found to be 127.8 ± 1.99 mp.

4.3.PH Value: The PH is another important parameter of nano emulsion. The excipients used in the formulation decide the pH of the final preparation and hence the route of administration. The pH of the formulations was measured using by digital pH meter. Results were taken in triplicate and the average was taken in to consideration. The estimated Ph value was found to be 5.3.

4.4. Drug content: The drug content of Drug nano emulsion formulation was measured using UV visible spectroscopic method. The 10 mg/ml of a liquid was prepared using nano emulsion formulation using diluting solvent. The samples were measured as 278.2 nm using UVVIS spectroscopic method. Results were taken in triplicate and the average was taken in to consideration. The results were found to be 9.87-9.98 mg/ml of the nano emulsion.

4.5. Centrifugation: This parameter was characterized to check the physical stability. The nano emulsion system was centrifuged at 5000 rpm for 10 minutes to determine whether the system shows signs of creaming or phase separation. The system was observed visually for appearance and the result was found to be there is no any phase separation or creaming.

4.6. Conductivity: Electrical conductivity of formulated samples was measured using a digital conductometer at ambient temperature. Results were taken in triplicate and the average was taken in to consideration. The estimated conductivity value was found to be 0.051 ms.

4.7. Dilution test: If the continuous phase is added in nano emulsion, it will not crack or separate into phases. Maximum amount of water and oil were added to o/w and w/o formulations respectively and then inspected visually for clarity and phase separation. Here 50- and 100-times aqueous dilution of the formulation was visually checked for phase separation and clarity. Results were taken in triplicate and the average was taken in to consideration.

4.8. Refractive Index: The refractive index of the drug loaded formulation was estimated on Rudolph Refractometer evaluated refractive index of the optimized formulation in triplicate. The Refractive index of F2 was found to be 1.501 ±0.0008.

4.9. Stability of Drug Nano emulsion: Samples of Drug nano emulsion formulations was sealed in ampoules and then placed in Stability chambers at different temperature conditions i.e., room temperature (25^0C) and accelerated temperature (40 ± 2^0C) for 2 months. Duplicate samples were withdrawn at 0, 1 and 2 months to evaluate their physical and chemical stabilities. The physical stability was evaluated by visual inspection for physical changes (such as phase separation and drug precipitation), and a globule size analyzer was used to determine the mean globule size and zeta potential after dilution with water. Chemical stability was expressed as the content of Drug

determined by UV visible spectroscopic method at 266 nm. Finally, the results were found that the formulation is stable both physically as well as chemically.

4.10. Light microscopy: Small amounts of the solution was taken and was spread on a glass slide, and then examined for the vesicle structure and the presence of insoluble drug crystals using ordinary light microscope with varied magnification powers (4x and 10x). Photomicrographs were taken using a digital camera (Sony Cybershot DSC-W690 16.1 megapixel, Tokyo, Japan). Each experiment was carried out in triplicate.

4.11. Fourier transforms infrared (FTIR) spectroscopy: Pure fluconazole, blank and optimized fluconazole formulation (F3) was analyzed using FTIR spectrophotometer. FTIR spectra of pure fluconazole showed an intense peak at 3149.44 cm^{-1} which was narrated to -NH stretching. Another, three distinct peaks were observed at 1093 cm^{-1} ,1461 cm^{-1} and 1740-3007 cm^{-1} which are convey to -C=O vibration, -COOH group and -CH stretching. All these peaks were narrated to evaluate any interaction between drug and excipients. But in case of optimized formulation (F_3), these four peaks were existing without any tangible shifting but with a slight amend.

5. SUMMARY & CONCLUSION

The present study was to design, formulate and evaluate of a nano emulsion formulation with using fluconazole as an active Pharmaceutical ingredient and by adding different kind of surfactants as well as cosurfactants in different ratios. The formulation was prepared by Aqueous titration method. The Physicochemical characteristics like FTIR studies, Drug contents studies performed to understand the solid-state properties of the drug. The results of the studies confirmed that FTIR studies provide ideas about evaluation of the compatibility between fluconazole and excipients. In this study the FTIR spectroscopy were successfully employed to assess the compatibility of fluconazole with the excipients used in the development of nano emulsion drug delivery formulation. Therefore, no definite interaction was observed between fluconazole and the excipients used in the development of in-vitro formulations of nanoemulsion drug delivery system of fluconazole. Another evaluation test like PH values determination, viscosity measurement, Centrifugation, conductivity, dilution, refractive index studies were performed to understand the drug loaded state properties of the Nano emulsion. From the above result concluded that Sunflower oil based nanoemulsion drug delivery system can be effective for Oral application in the treatment of fungal disease. From the above results it was concluded that the water insoluble drug, fluconazole was incorporated into the Sun flower oil Based Nanoemulsion Drug Delivery System

(SNDDS) with satisfactory physicochemical properties as well as stabilities studies that are suitable for further processing into either tablets or capsules.

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