FORMULATION AND EVALUATION OF NANOPARTICLES OF ITRACONAZOLE AND INCORPORATION INTO SHAMPOO TO TREAT SCALP INFECTIONS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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PHARMACEUTICS

By

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PUNJAB-144411

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Dedicated

To

My Family, brother,

Friends, and to my guide

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LIT (Pharmacy)/Department of Pharmaceutical Sciences

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PROPOSED TOPIC : Formulation and Evaluation of nano particles of Itraconazole and incorporation into shampoo to treat scalp infections

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	**Parameter**	**Rating (out of 10)**
1	Project Novelty: Potential of the project to create new knowledge	6.00
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	6.00
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	6.50
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Final Topic Approved by PAC: **Formulation and Evaluation of nano particles of Itraconazole and incorporation into shampoo to treat scalp infections**

Overall Remarks: Approved

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The expertise in this study belongs to those acknowledged above. All errors are mine.

Date: 29/04/2013 **Nuni Sagar**

Place: Jalandhar

Statement by the candidate

This is to submit that this written submission in my project report entitled **"Formulation and Evaluation of Nano Particles of Itraconazole and Incorporation into Shampoo to Treat Scalp Infections"** represents original ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

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The work described in this project report entitled **"Formulation and Evaluation of Nano Particles of Itraconazole and Incorporation into Shampoo to Treat Scalp Infections"** has been carried out by **Nuni Sagar** under my supervision. I certify that this is his bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

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ABSTRACT

The drug profile with poor solubility, toxicity, instability, incompatibility and poor penetration limits the physiochemical and pharmacokinetic performance of the drug molecule. Itraconazole the drug of interest belongs to the category of antifungal agent which shows hydrophilic character and is being used in the study extensively in treatment of various fungal infections. But, the major dispute is related to its side effects when administered orally and parentally, which on the other flip hammer its effectiveness in management antifungal therapy. For an instance, the oral capsule administration is associated with the Hepatobiliary disorder like Hyperbilirubinemia, based on the various clinical trials undertaken. Therefore, in the present study a multidisciplinary approach has been carried out in formulating the Solid lipid nanoparticles for topical delivery in the form of shampoo for scalp counteract the preexisting side effects. The objective behind development of SLNs was due to numerous reporting through researchers related to its effecting affinity towards stratum corneum and subsequent important in bioavailability. The approach is empowered by articulating from oral to tropical formulation and in addition, specific amendment in the functional components. It has been proposed through work that Itraconazole loaded nano particle will work onto overcome the side effects in a healthier way. Therefore, the presenting work highlights the improved delivery of Itraconazole to the specific site through encountering the two elementary approaches which can be proven as challenging and can dethrone the former side effects.

Keywords: Itraconazole, Solid Lipid Nanoparticles, Shampoo, Antifungal, Solubility

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AR	Analytical Reagent
AUC	Area under the curve
AFM	Atomic force microscopy
BCS	Biopharmaceutics Classification System
COA	Certificate of analysis
CCD	Central Composite Design
cm	Centimeter
cm^{-1}	Centimeter inverse
cP	Centipoise
°C	Degree Centigrade
DSC	Differential scanning colorimetry
DoE	Design of Experiment
3-D	3-Dimensiona
% EE	Percentage Entrapment Efficiency
et al.	And co-workers
FCCD	Face Centered Cubic Design
FDA	Food and Drug Administration
Fig.	Figure
FTIR	Fourier Transform Infra Red
g	Gram
mg	milligram
HCl	Hydrochloric acid
ICH	International Conference on Harmonization
KBr	Potassium Bromide
LCM	Liquid Crystalline Matrice
LOD	Limit of Detection
LOQ	Limit of Quantification
LQC	Lower Quantity Control
LR	Laboratory Reagent
λ	Lambda
Ltd.	Limited
µg	Microgram

min	minute
ml	millilitre
MQC	Middle Quantity Control
N	Normal
OPS	Optimized shampoo
% RSD	Percent Relative Standard Deviation
PI	Polydispersity Index
rev/mi	Revolutions per minute
rpm	Rotations per minute
RSM	Response Surface Methodology
SDA	Saboraud Dextrose Agar
UV	Ultra Violet
SLNs	Solid Lipid Nanoparticles
ITZ	Itraconazole
HPH	High pressure homogenization
W/O	Oil in water
O/W	Water in oil
W/O/W	Water in oil in water
nm	Nanometer
PGSS	Particles from gas saturated solutions
RESS	Rapid expansion of supercritical carbon dioxide solutions
PCS	Photon correlation spectroscopy
LD	Laser diffraction
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
FFEM	Freeze fracture electron microscopy
SLC	Static light scattering
NMR	Nuclear magnetic resonance
Pka	Partition coefficient
XRD	X-ray differaction
MLRA	Multiple liner response analysis

CHAPTER 1
INTRODUCTION

1.1 Drug delivery systems:

There are several problems associated with drugs depending upon their physicochemical and pharmacokinetic performance such as poor solubility, toxicity, instability, incompatibility and poor penetration (Patel *et al*., 2011). Due to which, it appeals for the development of suitable drug delivery system to overcome the barriers and to deliver the drug conveniently. Considering the fact, several conventional methods like micronization, precipitation, drug coating, use of surfactants etc. are used to conquer the challenges, but some limitations of these methods edged to the emergency of novel vesicular systems (Pallavi *et al*., 2006). Therefore, extensive efforts have been made to develop and modify the vesicular drug delivery systems to accomplish the desired goals. Numerous vesicular systems such as liposomes, niosomes, transferosomes and pharmacosomes have originated as promising approaches for better delivery of hydrophilic and hydrophobic drugs (Gande *et al*., 2007). The records and findings depict that these systems have presented better outcomes as per the need of the study. There were many advantages and approaches to accomplish certain goals, associated with the above mentioned developed drug delivery systems (Elnaggar *et al*., 2011). As entioning about the response broadly, These systems are capable of suggesting correct location for targeting, have offered protection from degradation of drug, controlled drug release along with brilliance in many other aspects (Biju *et al*., 2006., Gangwar *et al*., 2012).

Although, the modified drug delivery systems have certain advantages over the conventional systems but, the boundaries persists for these systems too. As generally these systems (liposomes, transferosomes and pharmacosomes) are containing phospholipids which have the affinity to get hydrolysed or oxidized offering instability during storage. The higher cost of the lipids also leads to overall enhanced price of such system (Chonn *et al*., 1995). Further researches envisaged, development of niosomes as a system which incorporate non-ionic surfactants as the component and are cost effective than other vesicular systems. But, again this system has also been found to produce various stability issues such as fusion, aggregation, sedimentation and leakage on storage (Kazi *et al*., 2010). Thus, the search for an effective drug delivery system to surpass all

such problems keeps undergoing. It is an indicative of vast expanse for enlargement of new and better delivery systems which can eliminate the difficulty related to stability, safety, efficiency, capacity and cost (Bhaskar *et al*., 2009). In the current study an effort has been provided to develop Itraconazole loaded nano suspension to overcome problems presented by conventional drug delivery systems.

1.4 Solid lipid nanoparticles for topical delivery

Solid lipid nanoparticles are composed of a high melting point lipid as a solid core coated with an aqueous surfactant and the drugs used are generally of BCS Class II and IV with solubility-related problems (Neha *et al*., 2013). In recent years, SLNs have been studied as a potential carrier for oral, intravenous, ocular, dermal, nasal, buccal, and vaginal drug delivery system. SLNs employ use of liquid lipids which safe, stable, and biodegradable in nature (Madan et al., 2014). SLNs have several advantages not only oral but for the topical delivery as well inducing drug penetration into the skin with reduced systemic exposure and less irritation (mangesh et al., 2009).

The drug delivery through the transdermal route has many advantages for administration of the drug in local therapy. Where the drugs are applied topically, the stratum corneum is the main barrier for the percutaneous absorption. The authorization of site-specific delivery to the skin is due to the small size and relatively narrow size distribution. SLN offers a high affinity toward the stratum corneum and consequently an improved bioavailability of the encapsulated material to the skin is reached (Wissing et al., 2003). With the explored facts and possibilities, the focus has now been increased for using SLNs in the cosmetic and topical product (Neeta et al., 2013). This can act as a topical carrier for topical delivery of numerous drugs including clotrimazole, prednicarbate, and betamethasone 17-valerate furthermore, the system has also been proven to posess high skin-targeting action (maia et al., 2000). In the present study, an attempt has been made to develop the SLNs by optimizing the components and formula and incorporation in to shampoo base to provide effective antifungal therapy for scalp. The study is based up on the investigation of appropriate components, method of preparation for development followed by the characterization and evalution of the medicated shampoo to offer improved antifungal action on scalp.

1.3 Problems associated with Itraconazole:

Itraconazole is a hydrophilic triazole antifungal agents used in the treatment of systemic and superficial infection. It has been extensively used as a first line agent to treat various fungal infections such as aspergillus in which fluconazole is not active. It is also prescribed for certain infections such as aspergillosis, candidiasis, and cryptococcosis in which other fungal agents are inactive (Sarkar et al., 2011). Oral and parenteral administration of the drug is associated with various side effects a headache, nausea, vomiting, diarrhea, dizziness, stomach upset, and muscle weakness. Itraconazole is also identified to have some rare side effects such as hepatotoxicity, liver failure but only with incersive doses and due to oral route of administration. In some cases adverse drug reaction has also been reported with oral capsules in clinical trails indicating Hepatobiliary Disorders like hyperbilirubinemia (Rao et al., 2009). Moreover, invasive parenteral delivery leads to poor patient compliance. Therefore, change of route of administration from oral to topical can serve the purpose to overcome the route related limitations. Furthermore, literature review has revealed less absorption of the drug through skin because of its hydrophilicity as compared to other drugs of its class. Thus secondly development of system with improved topical permeation by altering functional components would also a challenge for the study. Hence, the primary objective of the research is to design shampoo of Itraconazole containing its solid lipid nanoparticles for efficient delivery of the drug in order to treat the topical fungal infections.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Solid Lipid Nanoparticles (SLNs)

Introduction of solid lipid nanoparticles (SLN) in 1991 provided an alternative to the traditional carrier systems such as emulsions, liposomes and polymeric micro and macro-particles (Ekambaram *et al*., 2011). SLNs are composed of a high melting point lipid as a solid core coated by aqueous surfactant and the drugs used are generally of BCS Class II and IV with solubility related problems (Neha *et al*., 2013). Solid lipid particulate system is gaining more consideration as novel colloidal drug transporter for intravenous applications. These particulate system is sub-micron colloidal transporters (50–1000 nm), which are self-possessed of physiological lipid, distributed in water or in aqueous surfactant solution. It has various advantages over traditional colloidal carrier system such as less size, higher surface area, effective drug loading and capacities. (Ekambaram *et al*., 2011).

Solid lipid has provided a solution to the problems associated with liquid-liquid system, the surface and structure is shown in fig.2.1 (Ekambaram *et al*., 2011). From the various researchers, it has been found that the solid lipid supports a better network formation and frame as compared to the fluid lipids. The use of solid lipid as a m3er1atrix material for drug delivery remains very well recognized as lipid pellets for oral drug delivery (eg. Mucosolvan® retard capsules) (Muller RH *et al*., 2000). Broadly lipid includes triglycerides, partial glycerides, fatty acids, hard fats & waxes. As the lipid matrix is made from physiological lipids it decreases the danger of acute and chronic toxicity. The use of solid lipid at the place of liquid lipid is favorable as it has been exposed to increase control in excess of the release kinetics of encapsulated compounds and to improve the stability. Surfactants are added in order to stabilize the system. These are used as emulsifier to form o/w type emulsion and stabilizer for SLNs spreading and their optimal influenced by mainly the route of administration. SLNs are mainly prepared by high pressure homogenization or micro emulsification. There have been instability problems reported mainly because of hydrolysis reactions which can be overcome by

converting it into solid dry powders through lyophilisation and spray drying technique (Neha *et al*., 2013).

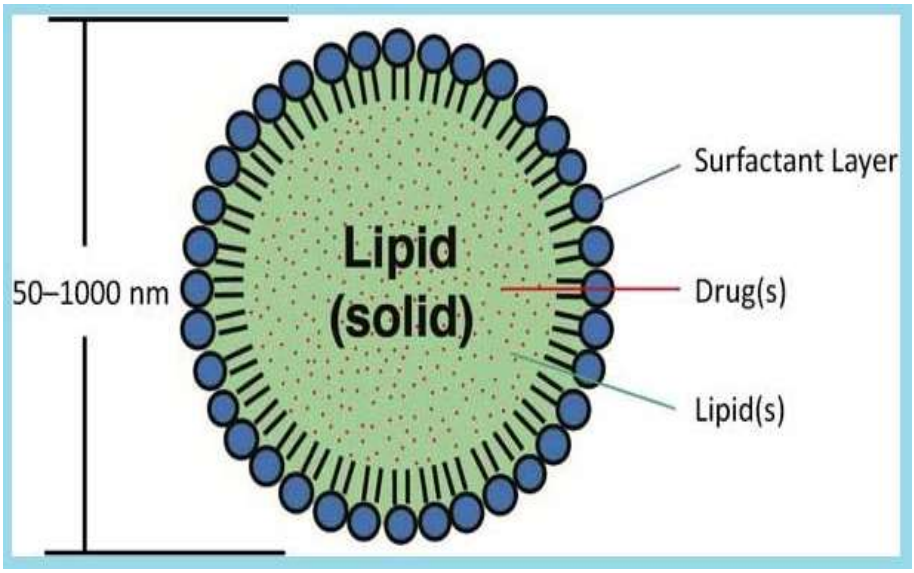


Fig. 2.1: Shows structure of Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) is one of the best available solution to overcome the dissolution problems of aqueous soluble drugs. SLNs syndicate all the benefits of polymeric nanoparticles, fat emulsions and liposomes. The diagrammatic representation of particulate drug transporters such as emulsions and liposomes are compared with SLNs in Fig. 2.2. (Ekambaram *et al*., 2011).

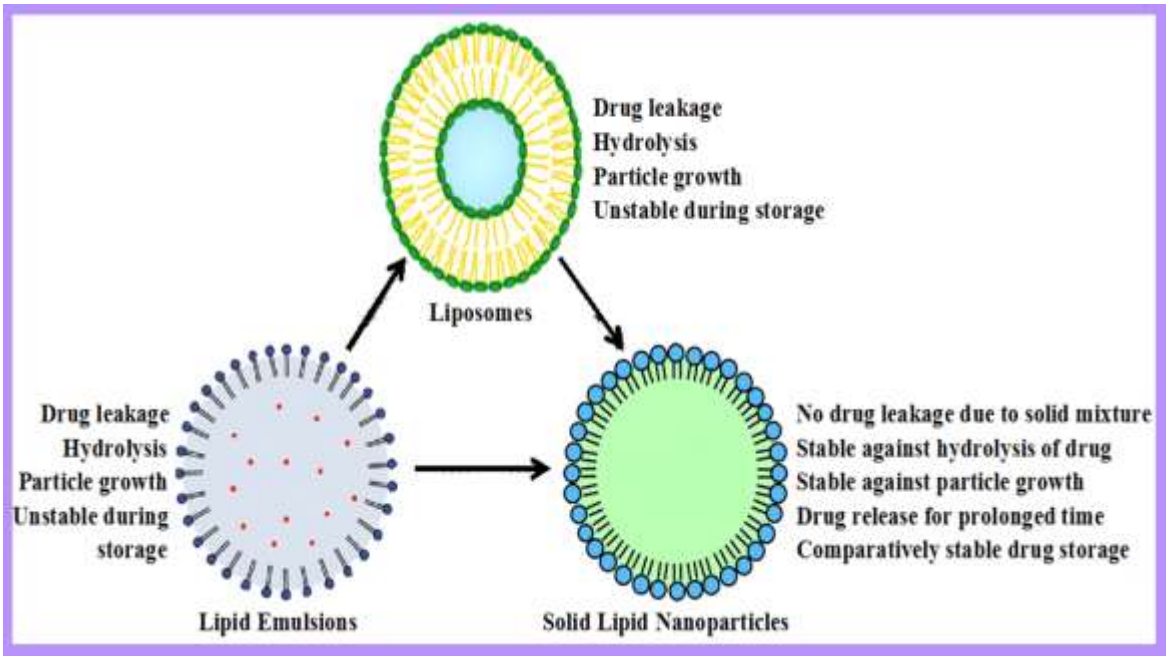


Fig. 2.2: A diagrammatic representation on SLN over emulsions and liposomes

2.1.1 Advantages of SLN (S. Mukherjee *et al*., 2009; Wolfgang Mehnart and Karsten Mader., 2001**)**

- SLNs are used for targeted and / or control drug release systems
- These offers better biocompatibility due to the lipid content
- They provide increased stability to the pharmaceuticals
- Enhanced bioavailability of entrapped bioactive mixtures have been reported
- Considerably stress-free to be produced than biopolymeric nanoparticles
- Does not require special solvents for formulation

2.1.2 Disadvantages of SLN (Melik *et al*., 2007**)**

- Particle growth which may lead to stability problems
- Sometime burst release is offered

2.1.3 Applications (Indu *et al*., 2008**)**

- Can offer controlled drug release and drug targeting
- offers increase drug stability
- ability to provide high drug payload
- No bio-toxicity of the carrier has been reported till date, which means it is biologically safe
- Capability for incorporation of lipophilic and hydrophilic drugs
- No problems with respect to large scale production and sterilization has been reported
- The system can offer increased bioavailability of entrapped bioactive compounds

2.2 Methods of preparation of solid lipid nanoparticles (Antoni *et al*., 2007**)**

1. High pressure homogenization
   A. Hot homogenization
   B. Cold homogenization
2. Ultrasonication/high speed homogenization
   A. Probe ultrasonication
   B. Bath ultrasonication
3. Solvent evaporation process
4. Solvent emulsification-diffusion method
5. Supercritical fluid method
6. Microemulsion based method

7. Double emulsion method
8. Spray drying method
9. Precipitation technique
10. Film-ultrasound dispersion
11. Solvent injection technique

2.2.1 High pressure homogenization (HPH)

The process of homogenization involves impulse of a fluid with high pressure (100–2000 bar) across a restricted opening (in the scope of a limited microns). The fluid quickens on a very short separation to high speed (over 1000 Km/h). Due to this high shear stretch and cavitation strings upset the particles depressed to the submicron range. For the most part 5-10% lipid substance is utilized but in some other studies 40% lipid content has also been studied (Ekambaram *et al*., 2011).

Hot homogenization and cold homogenization are the two general methodologies, which is loaded up on the idea of mixing the drug in main part of lipid to dissolve (Annette *et al*., 1998).

2.2.1.1 Hot homogenization:

Hot homogenization is done at temperatures above the melting point of the lipid. From high-shear mixing device the aqueous emulsifier stage (same temperature) is achieved through a pre-emulsion of the drug charged lipid melt. The process of HPH of the pre-emulsion is performed at the temperature above the melting point of the lipid. The decrease in viscosity of the inner phase represents the lower particle sizes due to higher temperature. The higher temperatures results in increasing the degradation level of the drug and the transporter. When enlarging the homogenization force or the sum of cycles may leads to enlarging in the particle size for the reason that kinetic energy of the particles get increased and they accumulate together (Dong *et al*., 2003).

2.2.1.2 Cold homogenization

To rectify the problems associated with hot homogenization such as: Temperature-prompted drug mortification, drug circulation into the aqueous phase during homogenization, the Cold homogenization method has been developed. In this process the drug holding lipid melt is chilled, the solid lipid is crushed to lipid microparticles and these lipid microparticles are distributed in an icy surfactant solution to achieve a pre-suspension. After obtaining the pre-suspension then it is homogenized at or under room

temperature, the gravitation strength is resilient enough to convert the lipid microparticles into solid lipid nanoparticles (Wei *et al*., 2008).

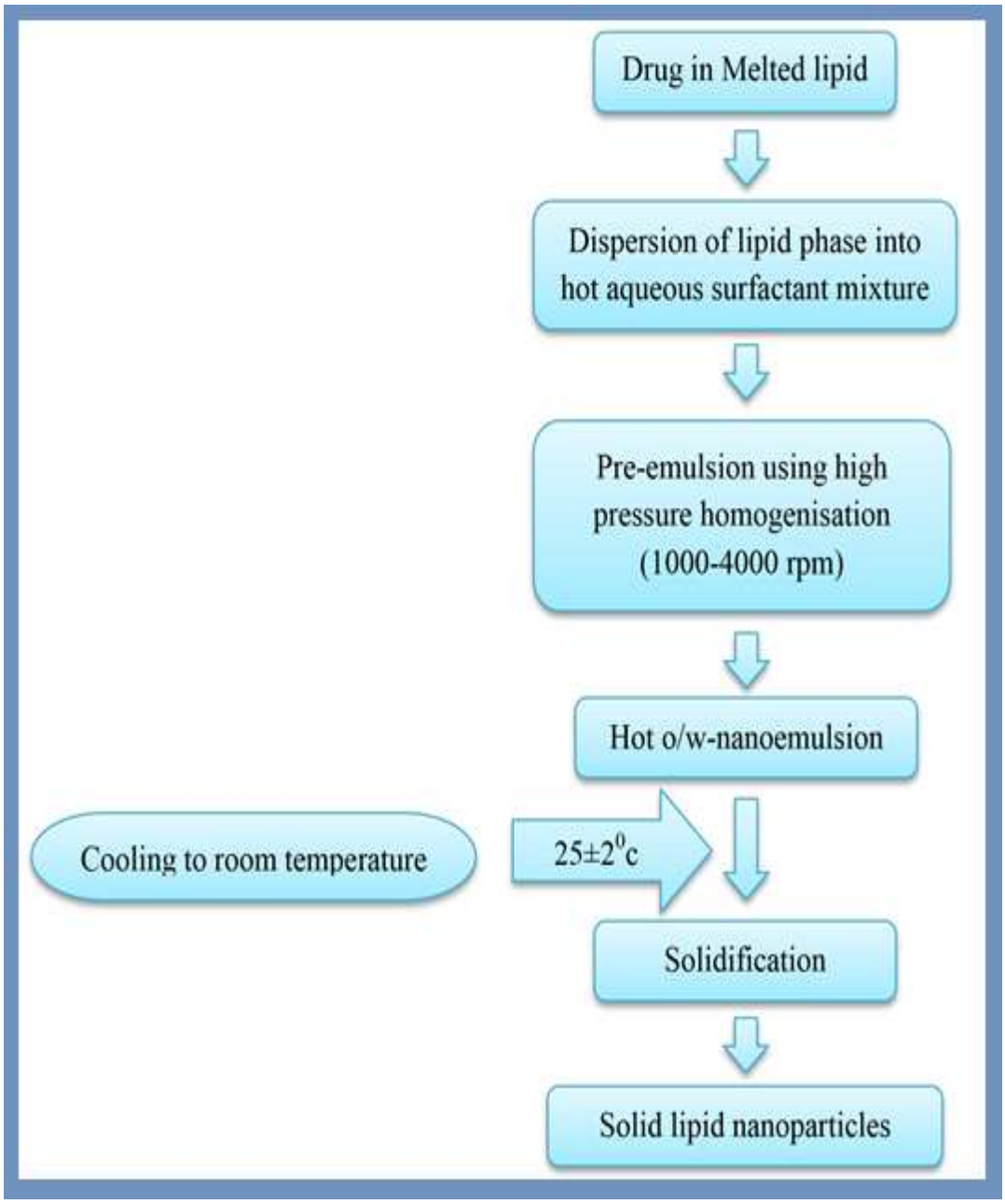


Fig. 2.3: Preparation of solid lipid nanoparticles by hot homogenization process

Advantages

- It requires low capital cost when compared to other methods
- This method can be validated at lab scale

Disadvantages

- This method demands more energy during the process

- It is demonstrated at lab scale due to this there may be a chance of biomolecule damage

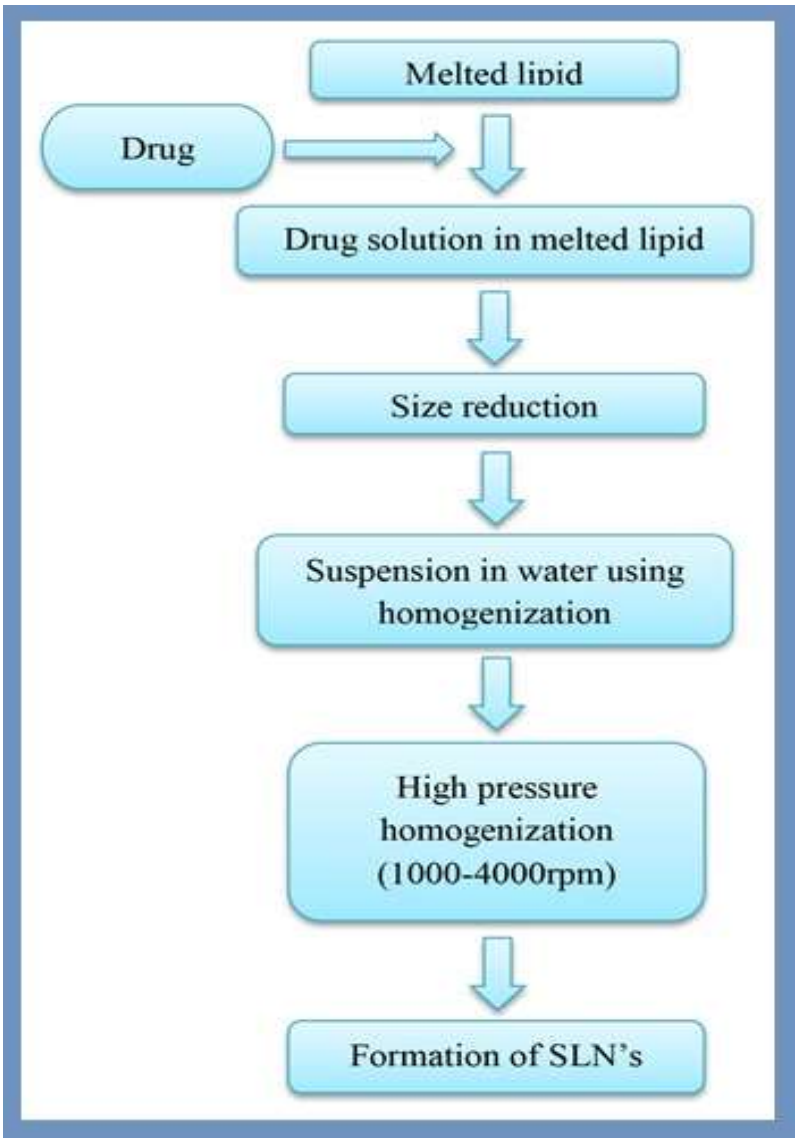


Fig. 2.4: Preparation of solid lipid nanoparticles by cold homogenization process

2.2.2 Ultrasonication/high speed homogenization

An ultrasonication or high speed homogenization procedure is also used for the creation of SLNs. For the formation of smaller particle size the mixture of both ultrasonication and high speed homogenization is necessary (Vivek *et al.*, 2007). During this process the shear stress can be reduced but, it is having some disadvantages like potential metal adulteration, physical instability like particle development upon storage. Therefore in the preparation process probe sonicator or bath sonicator is used (Neha *et al.*, 2013).

Advantages

- It reduces shear stress through the preparation process

Disadvantages

- During the process there are chances of metal adulteration
- Physical instability like particle development upon storage

2.2.3 Solvent evaporation

Solvent evaporation method can also be preferred to create SLNs. Initially The lipophilic material is solidified in a water-immiscible organic solvent (e.g. cyclohexane) then that is mixed with aqueous phase and the solvent is evaporated the nano dispersion is designed by precipitation of the lipid in the aqueous medium by assigning the nanoparticles of 25 nm mean size. Further, with the help of high pressure homogenization the solution is emulsified in an aqueous phase. Finally the organic solvent is removed from the emulsion by the help of evaporation under reduced pressure (40–60 mbar) (Biswajit *et al*., 2010).

Advantages

- In this process the particles can be easily reduced in size
- Highly mature technology and continuous process

Disadvantages

- This process required extensively high energy then other methods
- During this process there are chances of Biomolecule injury

2.2.4 Solvent emulsification-diffusion process

By using this technique, particles can be abtained with average diameters of 30-100 nm. During the preparation, the voiding of heat is the most important aspect and advantage of this technique (Ekambaram *et al*., 2011). The lipid is generally liquefied in the organic phase in water bath at 50 °C and to adjust the zeta potential an acidic aqueous phase is used in order to form coacervation of SLN, and then informal departure by centrifugation. The SLN suspension is rapidly produced and the entire dispersed system can then be centrifuged and re-suspended in distilled water (Neha *et al*., 2013).

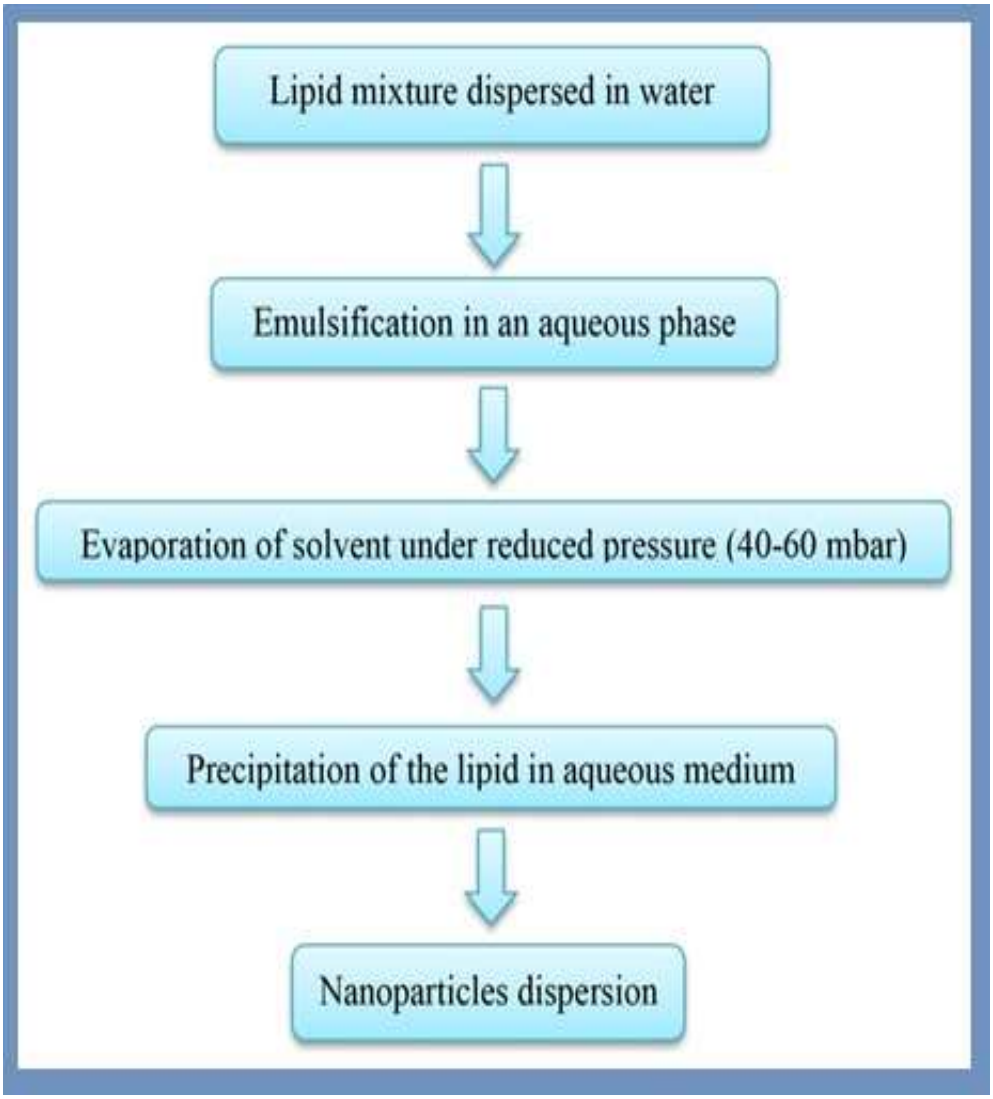


Fig. 2.5: Emulsification-diffusion method was systematically representation

2.2.5 Supercritical fluid method

This technique is a relatively new technique for production of SLN's (Shah *et al*., 2010). This is an another method for preparation of SLN's by particles from gas saturated solutions (PGSS) (Ekambaram *et al*., 2011). In this method the numerous variations are employed in platform technology for powder and nanoparticle formation. SLNs can be organized by the rapid expansion of supercritical carbon dioxide solutions (RESS) method. Carbon dioxide (99.99%) is used as an effective solvent for this method (Jawahar *et al*., 2011).

Advantages

- This process needs Mild pressure and temperature situations
- Carbon dioxide solution is the good optimal as a solvent for this process

2.2.6 Microemulsion based process

Micro-emulsions are two-phase systems which contains inner and outer phase (e.g. o/w microemulsions). In this process the continuous stirring is provided and an optically transparent mixture is obtained at 65-70°C, which logically self-possessed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water. The isolation of hot microemulsion is performed in icy water (2-3°C) under stirring. The preparation of solid product (tablets, pellets) by granulation method by using the SLNs dispersion can be done, but too much of water needs to be removed in case of low particle content. High-temperature gradients simplify speedy lipid representation and reduce aggregation. Due to the dilution step; probable lipid contents are significantly lesser when compared with the HPH based formulations (Vyas *et al*., 2002).

Advantages

- The process requires low mechanical energy
- The method is stabile

Disadvantages

- This method produces low nanoparticle concentrations

2.2.7 Double emulsion based method

The method involves the following steps: warm w/o/w double microemulsions can be formed by usig two steps. Initially, w/o microemulsion is prepared by addition of aqueous solution which is having drug in combination with melted lipid, surfactant and co-surfactant at a temperature slightly above the melting point of lipid to obtain a pure system. In another step, the microemulsion which is formed previously as w/o is added to a combination of water, surfactant and co-surfactant to attain a clear w/o/w system. SLNs can be formed by scattering the warm micro double emulsions in ice and then wash away with dispersion medium by ultra-filtration process. Multiple emulsions have inherent instabilities due to combination of the internal aqueous droplets within the oil phase, combination of the oil droplets, and disagreement of the layer on the surface of the internal droplets. If there should be an occurrence of SLNs creation, they must be steady for couple of minutes, the time between the arrangements of the reasonable double microemulsions and its dropping in cold aqueous medium, which is conceivable to accomplish (Chakraborty *et al*., 2009).

2.2.8 Spray drying method

It is an alternative and less expensive method to the lyophilization procedure. This suggests the utilization of lipid with a melting point more than 70^0C. The pre-eminent results were found with SLNs absorption of 1% in a solution of trehalose in aquatic or 20% trehalose in ethanol-aquatic combination. For the protection of the colloidal particle size during the spray dry the carbohydrates and low lipid content to be added. By using the ethanol-water mixtures instead of pure water may help to reduce melting of the lipid because of the preservation leads to small and heterogeneous crystals, the lower inlet temperatures (Helgason *et al*., 2009).

2.2.9 Precipitation technique

Solid lipid nanoparticles can also be formed by a precipitation technique which is categorised by the need for solvents. Dissolving the glycerides by using organic solvent (e.g. chloroform) and emulsification of the solution in an aqueous phase is overview of the method to be adopted. After disappearance of the organic solvent the lipid will be precipitated establishing nanoparticles (Maravajhala *et al*., 2011).

2.2.10 Film ultrasound dispersion technique

The drug and the lipid are placed into appropriate organic solutions, later on decompression, alternation and disappearance of the organic solutions, a lipid film is moulded, then the aqueous solution which contains the emulsions are added. Utilizing the ultrasound with the probe to diffuser at previous, the SLNs with the minute and unvarying particle size is formed (Loxley *et al*., 2009).

2.2.11 Solvent injection technique

It is a novel methodology to formulate SLNs, which has subsequent advantages over other production approaches like use of pharmacologically suitable organic solvent, easy handling and fast production method without technically sophisticated equipment. It is constructed on lipid precipitation from the liquefied lipid in solution. In this preparation, the solid lipid is liquefied in water-miscible solvent (e.g. ethanol, acetone, isopropanol) or a water miscible solvent mixture. Then this lipid solvent combination is injected through an injection needle into stirred aqueous phase with or without surfactant. The resulting dispersion is then filtered with a filter paper in order to eliminate any excess lipid. The presence of emulsifier within the aqueous phase supports the formation of lipid droplets at

the site of injection and stabilizes SLNs until solvent diffusion is complete by falling the surface tension between water and solvent (Gonnet *et al*., 2010).

2.3 Secondary production steps

- Freeze drying (Lyophilisation)
- Spray drying

2.3.1 Freeze drying

In case of freeze drying of the sample, higher solid lipid nanoparticles are formed with an extensive size distribution due to occurrence of aggregates among the nanoparticles by using lipid matrices. In this method there is elimination of water, and process of aggregation among SLNs. The satisfactory quantity of cryoprotectant can be used as a protection by which there is a aggregation of solid lipid nanoparticles throughout the freeze drying progression (Shuyu *et al*., 2011).

2.3.2 Spray drying

Spray drying is unconventional technique for lyophilization in order to convert an aqueous SLNs formulation into a dry product. This process has been used specially for SLN formulation, and spray drying is low-priced as campare to lyophilization (Mehnert *et al*., 2001).

The lipids with melting range at temperature >70°C had been optional for spray drying.

2.4 Influence of excipients

2.4.1 Formulation variables and their effect on particle size

There is major effect of particle size on the physical stability, biofate of the lipid particles, and release rate of the loaded drug. Hence the size of the SLNs has to be measured within reasonable range. Fine formulated systems like liposomes, nanospheres and nanoparticles must display a fine particle size distribution in the submicron size range below 1μm permitting the definition of colloidal particles (Alaa *et al*., 2010).

The particle size of lipid nanoparticles is affected by various parameters such as composition of the formulation such as surfactant/surfactant mixture, properties of the lipid and the drug incorporated production methods and conditions such as time, temperature, pressure, cycle number, equipment, sterilization and lyophilization. Large particle size is obtained at lower processing temperature. The hot homogenization technique gives a smaller particle size, generally below 500 nm, and a narrow particle size

distribution as compared to cold homogenization. Mean particle size as well as polydispersity index (PI) values are reported to be reduced at increasing homogenization pressure up to 1500 bar and number of cycles (3-7 cycles) (Helgason *et al*., 2009).

2.4.2 Influence of the lipids

By using the hot homogenization method, it has been reported that with high melting lipid the average particle size of solid lipid nanoparticles starts increasing. Whereas the other critical parameter for the preparation of nanoparticle will be different for different lipids. These parameters include the lipid hydrophilicity (influence of self-emulsifying properties of the lipid, shape of the lipid and its surface area) and velocity of lipid crystallization (Robhash *et al*., 2009). Increasing the content of lipid over 5-10% resulting into larger particle size and increased particle size distribution in the most formulation.

2.4.3 Influence of the emulsifiers

The concentration of surface active agents/mixture of surface active agents affects the particle size of the solid lipid nanoparticles. From literature, it has been found that greater the surfactant/lipid ratio lesser will be particle size. The decrease in concentration of surface active agents results in greater particle size during storage condition. Surface active agents act by decreasing the surface tension between the particles, therefore, increasing the surface area (Behzad *et al*., 2010).

2.5 Drug incorporation and loading capacity

The average particle size of the SLNs, its entrapment efficiency, and size distribution vary with types of lipid (triglycerides, fatty acids, steroids, waxes etc), emulsification agents (anionic, cationic, not -ionic) and preparation method etc. (Yingchao *et al*., 2006).

2.5.1 Factors determining the loading capacity of the drug in the lipid are

- Solubility of the lipid in melted state
- Miscibility of drug and lipid in its melted state
- Physical and chemical structure of solid lipid matrix
- Polymorphic nature of lipid

The essential parameter to obtain sufficient entrapment efficiency is a high solubility of the drug in melted lipid. Basically, the solubility of the drug should be higher in lipid because the solubility of the drug in lipid decreases when it cool down and lowers

even more in solid lipid. To increase the solubility of the drug in lipid melt one can add solubilizing agents. In addition, by using lipid matrix containing mono and triglycerides increase the solubility of the drug. The chemical nature of the lipid is important because lipids which form a highly crystalline structure with perfect lattice leads to drug exclusion.

2.6 Estimation of incorporated drug

2.6.1 Entrapment efficiency

This is the essential parameter of the SLNs because it affects the release characteristics of the drug from its formulation. The amount of drug entrapped per unit weight of SLNs is determined by separating the unentrapped drug from SLN formulation. The separation can be carried out by different techniques such as centrifugation filtration, ultracentrifugation and or gel permeation chromatography.

2.6.2 Centrifugation filtration

Filters eg. ultra sort-10 or ultra-free-mc are used with centrifugation. The percentage encapsulation can be assessed by determining the amount of drug present in the supernatant after centrifugation of SLNs formulation or by dissolution of the sediment in an appropriate solvent and further analysis.

2.6.3 Principles of drug release

In general, the principles of drug release from the SLNs formulations are as follows:

- There is an inverse relationship between the release of the drug and its partition coefficient.
- Drug release from the nanometer size range is higher due to higher surface area.
- Sustain drug release can be obtained by mixing the drug homogenously in the lipid matrix. Drug release depends on the drug entrapment model and type of lipid used in the preparation of SLNs.
- Fast drug release depends on the crystallinity behavior of the lipid and high mobility of the drug in lipid. Crystallization degree and drug release are inversely proportional to each other.

Factors which contribute to faster drug release are a smaller size or high diffusion coefficient, large surface area, the low viscosity of the lipid matrix and short diffusion distance for the drug. It has been investigated that with smaller particle size there is an increase in the velocity of drug release.

2.7 Storage stability of SLNs

The physical properties of the SLNs formulation in storage for a long interval of time can be estimated by monitoring changes in the value of zeta potential, drug content, particle size, its appearance and viscosity of the formulation with the function of time. Other parameters such as temperature and light appear to be of essential partner for long term stability. The zeta potential should remain higher than 60mV for a stable dispersion.

- $4^{o}C$ - Most favorable storage temperature.
- $20^{o}C$ - Long term storage did not result in drug loaded SLN aggregation or loss of drug.
- $50^{o}C$ – at this temperature instant growth of particle.

2.8 *In vitro* and *ex-vivo* methods for the assessment of drug release from SLNs

A large number of drugs which are highly hydrophilic in nature are chosen to incorporate in SLNs.

Various methods which are used to study the *in vitro* release of the drug:

- Side by side diffusion cells with artificial or biological membrane.
- Dialysis bag diffusion technique.
- Reverse dialysis bag technique.
- Agitation followed by ultracentrifugation or centrifugal ultra filtration.

2.9 In vitro drug release

2.9.1 Dialysis tubing and Reverse dialysis

In vitro drug release can be determined by using dialysis tubing. In this method, the SLN formulation placed in pre-washed dialysis tubing which is further sealed hermetically. The dialysis sac then dialysis against an appropriate dissolution media at room temperature, samples are withdrawn from the media at regular time interval. The samples are then centrifuged and analyzed for drug content using suitable method

In this method, a number of small dialysis sacs which contain 1ml of dissolution medium are placed in SLNs formulation. The SLNs are then displaced into the medium.

2.9.2 Ex-vivo model for determining permeability across the gut

Ahlin et al. explained in the study about the passage of enalaprilat SLNs across rat jejunum (Alessandro *et al.*, 1998). In the exprement, the jejunum of rat (20 – 30 cm distal from the pyloric sphincter) was excised. Qing Zhi Lu et al. excised 10 cm long segments of duodenum (1 cm distal to pyloric sphincter); jejunum (15 cm to pyloric sphincter),

ileum (20cm proximal to cecum) and colon (2 cm distal to cecum) are rapidly cannulated and ligated on both sides used for permeability studies (Qing *et al.*, 2009).

2.10 Characterization of SLNs:

Characterization of SLNs is very important due to colloidal particles of the formulation. The important parameters which can be evaluated for SLNs are particle size, zeta potential, and degree of crystallinity (polymorphism), time scale of distribution processes, drug content, surface morphology and in-vitro drug release (Eldem *et al.*, 1991).

These parameters are evaluated by using different techniques like Photon correlation spectroscopy (PCS), laser diffraction (LD), Scanning electron microscopy (SEM), transmission electron microscopy (TEM), Atomic force microscopy (AFM) and freeze fracture electron microscopy (FFEM) (Speiser *et al.*, 1990).

2.10.1 Measurement of particle size and zeta potential:

The most effective techniques for measurement of particle size are laser diffraction and photon correlation spectroscopy. Coulter counter method can also be used for the measurement of particle size but now, it is rarely used because there is need of electrolyte in this method by which colloidal dispersion is destabilized. Therefore, other two methods are commonly in use.

PCS measures the fluctuations of intensity of light which is caused by movement of particles. This is the best method to find out the size of nanoparticles having size range from few nanometers to 3 microns but not effective to detect large size microparticles. Beside this technique, laser diffraction can also be used to visualize particles and measure the size. The main advantage of LD measurements is that it covers the broad size range from nanometers to lower millimetre range. But, there is asain problem of measurement of particle size with these two techniques if samples contain populations with different sizes. In order to overcome limitation, these additional techniques may be useful like light microscopy (Meyer *et al.*, 1992).

Zeta potential is also very important parameter for evaluation of solid lipid nanoparticles, which is calculated by zetameter. Zeta potential is a measure of the size of the lectrostatic forces or attraction/repulsion charge between particles and is a major parameter which affects the stability of the formulation. The value of zeta potential strongly affects the aggregation and dispersion of the formulation and can be practiced to increase the stability of emulsion, suspension, and colloidal dispersion

2.10.2 Photon correlation spectroscopy (PCS**)**

PCS records the variation in intensity of scattered light in microsecond time scale. The reason of variations is interference of light scattered by individual particles due to movement particles and these variations are quantified by autocorrelation fiction. This function is used in expontial with decay constant. This is being related to diffusion coefficient in which particle size is calculated from its coefficient. The advantage of PCS is speed of analysis and sensitivity (Yang *et al., 1999*).

2.10.3 Static light scattering (SLC**)**

SLC is the method in which scattered light is collected and depending up on the response obtained from electromagnetic spectrums size is determined as primary variable solid. The advantage of this method is its fast and rugged response compatibility. But, the drawback of this method is requirement of clean equipment and sophisticated handling and use than PCS.

2.10.4 Acoustic method:

In this method particles size is determined by measuring the attenuation of sound waves by physically relevant equations. Electric field generated by movement of charged particles under influence of acoustic energy is used to find out the surface charge (Muhlen *et al.,* 1998).

2.10.5 Nuclear magnetic resonance (NMR**)**

The size and qualitative nature of nanoparticles can be determined by NMR technique. It provided information of physicochemical properties of the nanoparticles.

2.10.6 Electron microscopy:

Electron microscopy includes two methods for determination of particle size which are scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). It is the direct way to observe the nano ranged particle size. SEM is better for the physical characterization of nanoparticles. Whereas, TEM employed for determination and analysis of liquid sample (Jenning *et al.,* 2000)

2.10.7 Atomic force microscopy:

In this technique, a probe with atomic scale is placed between the sample which produces topological map due to the forces between the tip and the surface. Probe can be move in contact mode and the particles are then viewed and identified for the particle size ranges. Ultra high resolution is approachable by this technique (Drake` *et al.,* 1998).

2.10.8 X-ray diffraction and differential scanning calorimetric (DSC):

Degree of crystallinity can found by X-ray diffraction and differential scanning colorimetry (DSC). X-ray diffraction helps to identify the physical habit of the crystal whereas; DSC is also used to determine the nature and speciation of cryatallinity in nanoparticles through the measurement of glass and melting point temperature.

2.11 Sterilization of SLNs:

SLNs must be sterile for intravenous and ocular route of administration. High temperature during sterilization by autoclaving causes hot o/w emulsion in autoclave as results there is modification of size of hot nanodroplets (Yang *et al.,* 1999). But on slow cooling SLNs are reformed but some nanodroplets produces large size SLNs so to prevent this problem they are washed before sterilization and surfactant and cosurfactant are used in smaller quantities in hot systems (Wong *et al.,* 2006).

2.12 Oral lipid based formulations:

The studies have revolved many benefits of oral lipid based formulations for offering and improving physicochemical and physiological profiles of drug and dosage forms. Like improvement in GI absorption of poorly water soluble and lipophilic drugs and also to reduce the positive food effects. There are numerous oral lipid based formulations which are also follows like single component lipid solutions, self-emulsifying formulations (Lai *et al.,* 2006).

2.12.1 Excipients used in oral lipid based formulations:

Dietary oils composed of medium chain triglycerides like coconut and palm seed oils and long chain triglycerides like corn, peanut, sesame and soybean oils etc. It also include lipid soluble solvents like PEG400, ethanol, glycerine and acceptable surfactant are polysorbate 20, TPGS and span 20 (Pedersen *et al.,* 2006).

The total daily drug dose for administration of these formulations is less than 0.25µg and greater than 2000 mg the amount of drug in unit dose capsule varies in a range from 0.25µg to 500mg for oral solutions it ranges from 1µg/ml to 100mg/ml. Storage condition for oral lipid based formulations should be 2-8^{o}C for long term conditions (Rudolph *et al.,* 2004).

2.13 Work done on Solid Lipid Nanoparticles

Dong *et al.*, 2003: employed the modified high shear homogenization and an ultrasound technique to produce solid lipid nanoparticles. The mean particle size was measured by Laser Diffractometry (LD), Differential Scanning Calorimetry and X-Ray diffraction for measuring the interaction between drug and lipids. The Entrapment efficiency of SLNs were found to be more than 87% and showed long term physical stability as the leakage was very small after being stored for one month.

Mullar *et al.*, 2000: studied SLN for controlled drug delivery. The work described about various production techniques for SLN, drug incorporation loading capacity and drug release, especially focusing on drug release mechanisms. Relevant issue for the introduction SLN to the pharmaceutical market such as status of excipients, toxicity/ tolerability aspects, sterilization and long-term stability were also discussed. The potential of SLN to be exploiting for the different administration routes was highlighted. The different analytical tools for SLN characterization were also discussed.

Wolfgang *et al.*, 2001: studied the production, characterization and application of solid lipid nanoparticles. The selection of ingredients and different ways of SLN production (like-High shear homogenization and ultrasound, High pressure homogenization, Hot homogenization, Cold homogenization, solvent emulsification/evaporation and microemulsion) were described and practiced by the authors along with the applications of SLNs.

Wissing *et al.*, 2004: describe the use of nanoparticles based on solid lipid for parenteral application of drug. Different production methods included the suitability for large scale productions. Stability issue and drug incorporation mechanisms into the particles were discussed and the biological activities of parenterally applied SLNs were studied. Biopharmaceutical aspects such as pharmacokinetics profile as well as toxicity aspects were also reviewed.

Nguyen *et al.*, 2007: prepared nanoparticles loaded with ferrocenyl tamoxifen derivatives for breast cancer treatment. The organometallic compounds for were used in the study binding to hydroxytamoxifen for more stable metal ligand and also to enhance cytotoxicity of hydroxytamoxifen, which increased the lipophilicity of the compound to facilitate its passage through the cellular membrane. The encapsulation efficiency was determined by UV-VIS spectroscopy and the concentration of FC-dioH and Diclofenac

diethylammonium (DFO) in the aqueous phase after centrifugation was determined by the absorbance at 304 nm for FC-dioH and 276 nm for DFO respectively.

Giacomo *et al.*, 2005: Solid lipid nanoparticles containing Tamoxifen for the characterization and *in vitro* antitumoral activity. Solid lipid nanoparticles (SLNs) containing tamoxifen, a nonsteroidal antiestrogen used in breast cancer therapy, were prepared by microemulsion and precipitation techniques. Tamoxifen loaded SLNs seem to have dimensional properties useful for parenteral administration, and *In vitro* plasmatic drug release studies demonstrated that these systems are able to give a prolonged release of the drug in the intact form. The potential application of tamoxifen-loaded SLNs as a carrier system at prolonged release was found to be useful for intravenous administration in breast cancer therapy.

Suh *et al.*, 2005: Prepared and evaluated solid lipid nanoparticles (SLNs) containing 5-Fluorouracil and its derivative. SLNs were prepared with lauric acid, as the lipid core. Tween 20 and tween 80 were used as surfactant by using 5-fluorouracil and 1-benzoyl-5- luorouracil were used as model drugs. Drug-loaded SLNs were prepared by the hot homogenization technique and evaluation includes analysis of the physical stability, entrapment efficiency of drugs as well as release profile. The particle size of SLNs was 40~600 nm. The recording also determined the speed of homogenization had positive effects on reduction of mean particle size of SLNs. In case of entrapment efficiency,it was found to be wore in case of 1-Benzoyl-5-fluorouracil 5-Fluorouracil. Furthermore, the study depicted that the higher surfactant concentration, had better has compared to release rate at the range of 1.5-2.5 %.

Anders *et al.*, 2004: Investigated the flow behavior of dispersions of solid triglyceride nanoparticles. A significant increase in dispersion viscosity was found in the triglyceride in the of sequence trimyristine < tripalmitine < tristerine. This effect can be clearly attributed to an increase in particles shape anisometry with increase in length of the lipid fatty acid chain. Surfactants, which were added during the crystallization of the dispersed lipid seems to have an additional effect on the nanoparticles shape.

Vobalaboina *et al.*, 2004: prepared and characterized clozapine loaded solid lipid nanoparticles to evaluate for the *in vitro* drug release kinetics. Solid lipid nanoparticle (SLN) delivery systems of clozapine was developed using various triglycerides (trimyristin, tripalmitin and tristearin), soylecithin 95%, poloxamer 188 and charge

modifier stearylamine. *In vitro* release studies were performed in 0.1 N HCl, double-distilled water and phosphate buffer, pH 7.4, using modified Franz diffusion cell. The Stable SLN formulations of clozapine was found to posess mean size range of 60–380 nm and zeta potential range of −23 to +33 mV were developed. More than 90% clozapine was entrapped in SLNs which shows good entrapment potential of the system.

Michele *et al.*, 2003: prepared SLNs by solvent emulsification diffusion technique. Emulsification of butyl lactate for benzyl alcohol solution of solid lipid in an aqueous solution of different emulsifiers, was done followed by dilution of the emulsifiers with water, was used to prepared GMS nanodispersion was found with narrow size distribution. The combined use of two or more emulsifying agent appeared to produce mixed surfactant film at the interface having high surfactant coverage as well as sufficient viscosity to promote stability.

Bin *et al.*, 2006: prepared solid lipid nanoparticles of metoxantrone for local injection against breast cancer and its lymph node metastases by using film dispersion ultra sonication method and evaluated on mice. A thin film was prepared by dissolving various amounts of lecithin and compritol-888 in chloroform. Various parameters like encapsulation efficiency, drug content, *in vitro* drug release was found by changing the various concentrations of surfactants**.**

Tiyaboonchai *et al.*, 2007: studied the formulation and characterization of curcuminoids loaded solid lipid nanoparticles. The SLNs of curcuminoids were successfully developed using microemulsion technique.

Elena *et al.*, 2002: studied the incorporation of cyclosporine A in solid lipid nanoparticles. In the study the particle were prepared from warm o/w emulsion, by using stearic acid, phosphatidylcholin and taurocholate. Cyclosporine A loaded SLNs were characterized for SEM and in-vitro drug release.

Hu et al; 2002: prepared solid lipid nanoparticles with Clobetasol propionate by a novel solvent diffusion method in an aqueous systems and performed physicochemical characterization.

Alix *et al.*, 2003: studied Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) have been as method for the imaginary and characterization of SLNs formulated from amphiphilic cyclodextrins.

Zhang *et al*., 2006: prepared amorphous cefuroxime axetil nanoparticles by controlled nanoprecipitation method without surfactant. Prepared nanoparticles were evaluated for particle size distribution, fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), differential scanning calorimetry (DSC) and dissolution testing.

Pallavi *et al*., 2006: developed and evaluated topical formulation containing solid lipid nanoparticles of vitamin A. The nanoparticulate dispersion and gel were evaluated for various parameters like particle size, *in vitro* drug release, drug content, and *in vitro* penetration etc.

Kumar *et al*., 2006: prepared nitrendipine loaded solid lipid nanoparticles by using different lipids such as tripalmitin and glyceryl monostearate by various methods such as high pressure homogenization and solvent evaporation. Further the prepared SLNs were characterized by particle size, zeta potential measurement, crystallinity and *in-vitro* studies.

2.14 Fungal infections (Ringworm of the scalp)

Fungal infection has been divided into two major classes which are systemic and superficial categons (Bennet *et al*., 2001). Severe skin diseases are caused by fungal species known as fungal infection (Bseiso et al., 2015). Classification of antifungal drugs is given according to the chemical structure such as polyene antifungals, allylamine antifungals, azole antifungals, echinocandin antifungals and miscellaneous (Bennet *et al*., 2008).

Ringworm in the scalp is caused by fungal species. The name ringworm is due to the circular marks on the skin, with flat centers and raised border caused by fungus. The infection is also called as *Tinea capitis***,** an infection which affects scalp and hair shafts creating small patches itchy and scaly. Ringworm is a highly communicable infection, which spreads from person to person by using towel hats and sharing objectives like ringworms affects persons of all age but mostly children (Bseiso et al., 2015).

Some of the common signs of scalp ringworm are:

- The infection starts with severe dandruff on few parts of the scalp. Symptoms can be confused with psoriasis.
- Few infections cause bald patches on the scalp. These patches are mostly inflamed and scaly. Sometimes small broken hairs remain scattered on the patches with infection.

- In few cases, painful boils arise on the bald patches.
- In severe cases, a large 'boggy' swelling arises on the bald section of the scalp, known as kerion. This swelling is oozing and tender and should be treated early. If not treated fast it may lead to permanent hair loss from the area of infection.
- In the case of severe infection, this may lead to high body temperature and glands present in the neck may start swelling.

2.14.1 Sources of infection

Dermatophyte group of fungi cause an infection known as scalp ringworm. This group of fungi is categorized into three groups according to the area in which they usually developed. Geophilic organisms live in soil, zoophilic organisms on animals, and anthropophilic organisms on humans. In most of the cases of childhood ringworms from last twenty years have been spread by zoophilic organisms *Microsporum Canis* or *Trichophyton verrucose* (from cattle). Recently, the predominant organism has changed to an anthropophilic one, *T.Hondurans*, and it spreads directly from child to child. In Britain's larger cities this organism is responsible for most of the scalp ringworm. The reason behind this change is unknown.

2.15 Treatment for scalp ringworm

Griseofulvin has been used as a best antifungal agent for the treatment of scalp ringworm from 1950's. For the treatment of fungal infection 8-12 weeks, regular prescription of the griseofulvin is required. There is no liquid dosage form of griseofulvin is present in the market. So, in the pediatric patient is given in crushed form with a little amount of water.

Terbinafine is another drug which can be used in place of griseofulvin in the treatment of scalp ringworm. This drug is not licensed for the treatment of scalp ringworm but it is effection in this fungal infection. For this infection, the four-week course is required. To clear the infection completely, it is necessary to complete the course. Other drugs that can be used in the treatment Itraconazole and fluconazole.

Most of the antifungal agents are well tolerated and side effects are less as a comparison to another drug. But side effects are more common to some people and some people are highly sensitive to some people. For example, the women cannot get pregnant during the treatment with griseofulvin. Those men getting a regular prescription with

griseofulvin should use contraceptive due to deterioration of sperms. Therefore it is necessary to read leaflets provided with medicines to avoid the side effects.

Antifungal shampoo and cream preparation are unable to remove the infection completely. This is because the infection penetrates into te shaft where shampoo and cream are unable to penetrate. That is why they cannot clear the infection. Antifungal shampoos and creams are prescribed twice a week with other medication to treat the infection. For example, selenium shampoo, ketoconazole shampoo or terbinafine cream. This is to treat the infected area quickly and to decrease the chances to spread the infection.

2.16 Itraconazole: An antifungal agent

Itraconazole is a synthetic triazole antifungal agent. The drug is slightly white to yellow in color and is slightly soluble in alcoholic agents and highly soluble in methylene chloride. It is highly lipophilic in nature and insoluble in water with extremely baric nature, Pka value of 3.7 which is ionized at low pH value. It is a lipophilic antimycotic drug with three chiral centers. It is clinically used as the stereoisomeric mixture (Prasuna et al., 2013).

It is orally active triazole active against a wide spectrum of fungal species including dermatophytes, Malassesia furfur, Candida species, Aspergillus species, and Histoplasma capsulatum var. capsulatum. Mechanism of action of Itraconazole is quite clear as it interacts with 14-a demethylase, a cytochrome P-450 enzyme which converts lanosterol to ergosterol. The drug is available in various dosage forms and is administered through various routes of administration. Commonly the capsule dosage forms used to treat infection caused by fungal species in lungs that can spread throughout the body and can be used to treat the infection in fingernails. Capsules and tablets of Itraconazole are used to treat the fungal infection in toenails. The liquid dosage form of Itraconazole is also available to treat the yeast infection in mouth and throat or of the esophagus (Prasuna et al., 2013).

2.17 Work done on Itraconazole

Prasuna et al., 2013: described the analytical methods for quantitative determination and identification of pharmaceutical dosage form of Itraconazole and biological samples. The methods like colorimetry, spectrophotometric, spectroflorimeteric

and chromatographic techniques were discussed and the importance of these techniques was conclusion for analysis of Itraconazole.

Sampathi et al., 2014: discribed about the development of liposomal hydrogel complex drug delivery system of Itraconazole. Carbopole is used to make the hydrogel. The compatibility study was done by using FTIR. The evaluation of liposomal hydrogel complex drug in delivery system was done by finding particle size, zeta potential, encapsulation efficiency and for skin permeability. The results obtained from these evaluations proved that liposomes in hydrogel complex system drug permeability from skin and this drug delivery system offers better results to enhance the transdermal permeation Itraconazole for the treatment of topical infections.

Dileep et al., 2015: decribed the formulation and evaluation of Itraconazole loaded ethosomal gel which is prepared by cold method by using different polymers and carbopol. The evaluation of gel was done by different parameters like, pH measurement, organoleptic character sticks, drug content entrapment efficiency, drug release, and content uniformity etc. They concluded that Itraconazole ethosomes offers a promising approach of topical drug delivery system.

Deveda et al., 2010: studied the development of gellified emulsion for control delivery of Itraconazole by making emulsion and the incorporeity the emulsion in carbopol gel. The evaluation of these formulations was done on the basis of viscosity, drug content, pH, spreadability, in- vitro and stability studies. The microbial assay was done on rabbit. From the evaluation of different parameters the optimum results were obtained. The study was summarised and emulsion based system was more effective and for treatment of fungal disorders.

D.V.et al., 2012: the objective of research was to prepare and evaluate noisome of Itraconazole by applying factorial design. The evaluation of Itraconazole loaded niosome was done by evaluating the entrapment efficiency, vesicle size, drug release ,skin permeation and antimycotic activity and from the results it was concluded that niosomes offers larger zone of inhibition than marketed formulation. Therefore, Noisome are the better formulation for topical treatment.

Mohamad et al., 2013: The main objective of the research was to formulate and evaluate various polymeric films for the treatment of fungal infection. In this study, different films containing Itraconazole were prepared by using solvent casting technique.

Various physicochemical properties were tested like, physical endurance, tensile strength, elasticity, water vapor permeation and water loss the release drug from the film was also determined concluded that anti-fungal drugs like Itraconazole having broad spectrum the study concluded that can be successfully incorporated in polymeric film.

Parikh et al., 2010: described about the quantitative determination of Itraconazole by U.V spectrophotometric method. The evaluation of Itraconazole was done by different parameters like linearity, accuracy precision, LOD and LOQ were studied according to ICH guidelines. From these all parameters result was found that proposed method was precise, accurate and rapid.

Reddy et al., 2012: The aim of the research work for determination and analysis of sample drug was to prepare and assess 1% w/v corbopol gel consisting of solid-lipid nanoparticles by hot homogenization technique. The formulations were characterized for particle size, zeta potential, entrapment efficiency, DSC, FTIR and XRD studies. In-vitro and in vivo studies carried out by using franz diffusion cell for 24hours. The evaluation of gel was done by the conclusion recording the rheological behavior and permeation studies by using rats. A last concludes that SLN system is innovative carrier system for the treatment of fungal diseases.

Kumar et al., 2013: described about various techniques which are used to increase the solubility of hydrophobic drugs like physical modification, chemical modification of the drug. Other miscellaneous methods were also used to increase the solubility of the drug. Most of the drugs are lipophilic in nature, so different techniques are used to increase the solubility of lipophilic drugs.

Mukharjee et al., 2009: the objective of the study was to design and evaluate suitable size ranges Itraconazole solid lipid nanoparticles by using lipid and susfactant for the purpose of increasing the therapeutic activity and reduction of toxicity by using microemulsion dispersion method. These SLNs were characterized for particle size, zeta potential, DSC, IR and XRD analysis.

Modyala et al., 2014: provide information about the formulation and evaluation of Itraconazole lozengesfor oropharyngeal candidiasis. Lozenges were prepared by wet granulation technique using three different binders at different concentration and stability studies were carried out. The evaluation of this formulation was done by analyzing postcompression parameters by pharmaceutical standard method. Stability studies were

done according to ICH guidelines and microbial studies were also carried out to check the antimycotic studies. Stability indicated that formulations were stable for three months and formulation were successful in delivering the drug topically.

Kamble et al., 2014: Investigated the potential of emulgel for the enhancement of topical delivery of Itraconazole by using two types of gelling agents carbopol 934 and carbopol 940. The prepared formulations were evaluated by physical appearance, viscosity, drug release, skin irritation test, antifungal activity, TEM and stability. All prepared emulgels showed acceptable results and it was suggested that emulgel formulation shows good drug release as compare to creams.

Nakaraniet al., 2010: the purpose of the study was to formulate the nano suspension of Itraconazole to increase different parameters like solubility, dissolution and bioavailability of Itraconazole. Nano suspension was prepared by using pearl milling technique by using zirconium beads as a milling media. Characterization of the nano suspension was done by analyzing the particle size, drug content, SEM, DSC and XRD techniques.

Mohanty et al., 2013: described the in-vitro permeation characteristics of Itraconazole from oil drops and ophthalmic ointment through got and sheep corneas. Permeation from castor oil was found to be more than kardi oil. Atlas they conclude that permeation of Itraconazole from ointment containing drug was more conclusion was made that the castor oil shows maximum zone of inhibition against *candida albicans*.

Goyal et al., 2013: The aim of this research was to improve the dissolution and bioavailability of Itraconazole by its adsorption on calcium silicate employing adsorption method for preparation of solid dispersion. The evaluation was done by DSC and X-ray diffraction analysis. The conclusion of the study was increased dissolution rate of drug due to its crystallinity.

Prasad et al., 2010: described about the enhancement of dissolution and bioavailability by preparing the solid dispersion by using lipid material as inert excipient. The evaluation of the formulation was done by particle size, drug content, particle size distribution, drug excipient compatibility, in-vitro dissolution and in-vivo bioavailability.at last the result concluded enhance bioavailability through solid dispersion by increasing its aqueous solibility.

Patel et al., 2013: presented the potential of microemulsion formulation of Itraconazole prepared by using pseudophase ternary diagram. The evaluation of this microemulsion was carried out by various tests like zeta potential, size distribution study was evaluated for % transmittance, conductance and centrifugation the optimized microemulsion was incorporated in carbopol and subjected to various tests like extrudability, drug content, spreadibility, in-vitro dryg release and in-vitro antifungal activity angainst *C. albicans*.

Hire et al., 2007: The purpose of study was to formulate microemulsion gel for the topical delivery of Itraconazole for its antifungal activity by increasing penetration through skin with the help of pseudoternary phase diagram. The evaluation of the formulation was done by its recording rheological study, in-vitro permeation study and in-vitro antifungal activity. The study concluded that microemulsion based gel is better choice as vehicle for delivery of Itraconazole as topical antifungal agent for treatment of fungal infections.

Kumar et al., 2013: studied about the compatibility of antifungal drug Itraconazole with different excipients for the preparation of solid lipid nanoparticles. Differential scanning calorimetry, isothermal stress testing, Fourier transform infrared spectral analysis, optical microcopy, and X-ray powder diffraction analysis were performed for testing the compatibility between the drug and the excipients. Results of the study suggested that the stearic acid exhibited drug–excipient interactions, whereas all other excipients used in the study were found to be compatible with Itraconazole.

Sawant et al., 2015: The aim of the present research work was to investigate the potential of emulgel in enhancing the topical delivery of Itraconazole. Emulgel formulations of Itraconazole were prepared using two types of gelling agents namely: Carbopol 934 and Carbopol 940. Evaluation of prepared formulation was done by analysing physical appearance, viscosity, globule size, drug release, antifungal activity, TEM and stability and it will give the acceptable results.

Jacob et al., 2005: studied single dose pharmacokinetics of bioadhesive Itraconazole tablets in healthy volunteers. A single-dose, randomized, two-way crossover study in sixteen healthy volunteers was conducted at the Shandon Clinic, Ireland. Volunteers were dosed either with 100mg Sporanox® capsules or Spherazole™ Tablets 20 minutes after a standard breakfast. Blood samples were collected at appropriate

intervals and plasma Itraconazole and hydroxyItraconazole levels were determined by LC-MS/MS. Spherics bioadhesive formulation resulted in greater bioavailability than the Sporanox® capsules, in terms of Cmax and AUC, for both Itraconazole (parent compounds) and hydroxyl-Itraconazole (active metabolite). Analysis of the log transformed data demonstrated a 17% reduction in Cmax variability and 37% reduction in AUC variability based on coefficient of variation for Spherazole™ compared to Sporanox® capsules.

Mukherjee et al., 2009: studied design and evaluation of Itraconazole loaded solid lipid nanoparticulate system for improving the antifungal therapy. The microemulsion mediated nanoparticle preparation methodology ensured high drug loading (80%), low and narrow size distribution and provided a reproducible and fast production method. They studied feasibility and suitability of lipid based colloidal drug delivery system, employing optimized design to develop a clinically useful nanoparticle system with targeting potential.

Prasad et al., 2010: prepared and characterize Itraconazole solid dispersions for improved oral bioavailability. The formulations have demonstrated the significant improvement of bioavailability (AUC=14384ng/h/ml) compared to plain drug suspension (AUC=4384ng/h/ml). These results demonstrated the efficacy of solid lipid dispersions for the enhancement of oral bioavailability of Itraconazole by increasing its aqueous solubility

Madgulkar et al., 2008: studied formulation development of mucoadhesive sustained Release Itraconazole tablet using response surface methodology. The optimized mucoadhesive formulation was orally administered to albino rabbits, and blood samples collected were used to determine pharmacokinetic parameters. The solid dispersion markedly enhanced the dissolution rate of Itraconazole. The Bioadhesive strength of formulation was found to vary linearly with increasing amount of both polymers. Formulations exhibited drug release fitting Peppas model with value of n ranging from 0.61 to 1.18. Optimum combination of polymers were identified which provided adequate bioadhesive strength and fairly regulated release profile. The experimental and predicted results for optimum formulations were found to be in close agreement. The formulation showed C_{max} 1898±75.23 ng/ml, t_{max} of the formulation was 2 h and AUC was observed to be 28604.9 ng h/ml.

Shim et al., 2006: performed characterization of semisolid dosage forms prepared by hot melt technique. In addition, the distinctive functional peaks and chemical shifts of Itraconazole were well retained after processing into semisolid preparations. They concluded from the data that Itraconazole was stable during incorporation into semisolid preparations by the hot melt technique. In particular, Itraconazole semisolid preparations composed of polysorbate 80, fatty acids and organic acids showed good solubility and dissolution when dispersed in an aqueous medium. It was anticipated that the semisolid dosage forms would be industrially applicable to improve the bioavailability of poorly water-soluble drugs.

Punitha and Girish., 2010: reviewed polymers in mucoadhesive buccal drug delivery system. The mucoadhesive interaction is explained in relation to the structural characteristics of mucosal tissues and the theories & properties of the polymers. The success and degree of mucoadhesion bonding is influenced by various polymer-based properties. Evolution of such mucoadhesive formulations has transgressed from first generation charged hydrophilic polymer networks to more specific second-generation systems based on lectin, thiol and various other adhesive functional groups. The authors reviewed the mucoadhesive polymeric platforms, properties & characteristics to provide basics to the young scientists, which will be useful to circumvent the difficulties associated with the formulation design.

Ahmed et al., 2010: Formulate and evaluate gastric mucoadhesive drug delivery systems of Captopril. The alginate-cellulose acetate phthalate beads showed the better sustained release as compared to all other alginate polymer combinations. Regression analysis showed that the release followed zero order kinetics in 0.1 N hydrochloric acid (pH 1.2). The objectives achieved were formulation, evaluation and usefulness of sodium alginate mucoadhesive eads of captopril with different mucoadhesive polymers, findings of which can be applied for sustained delivery of drugs with mucoadhesion.

Hong et al., 2006: developed a new self-emulsifying formulation of Itraconazole with improved dissolution and oral absorption. The results demonstrated that the SEDDS of Itraconazole composed of Transcutol, Pluronic L64 and tocopherol acetate greatly enhanced the bioavailability of Itraconazole after the dose, particularly not influenced by food intake. Thus, the system may provide a useful dosage form for oral waterinsoluble drug without food effect.

Rabinow et al., 2007: studied Itraconazole I.V nanosuspension enhanced which the efficacy through altered pharmacokinetics in the rat. Their goal was to evaluate an intravenous Itraconazole nanosuspension dosage form, relative to a solution formulation, in the rat. Itraconazole was formulated as a nanosuspension by a tandem process of microcrystallization followed by homogenization. Acute toxicity, pharmacokinetics, and distribution were studied in the rat, and compared with a solution formulation of Itraconazole. Efficacy was studied in an immunocompromised rat model, challenged with a lethal dose of either Itraconazole-sensitive or Itraconazole-resistant *C. albicans*. Itraconazole nanosuspension was tolerated at significantly higher doses compared with a solution formulation. Pharmacokinetics of the nanosuspension was altered relative to the solution formulation. C_{max} was reduced and $t_{1/2}$ was much prolonged. The higher dosing of the drug, enabled in the case of the nanosuspension, led to higher kidney drug levels and reduced colony counts. Survival was also shown to be superior relative to the solution formulation. Thus, formulation of Itraconazole as a nanosuspension enhanced the efficacy of this antifungal agent relative to a solution formulation, because of altered pharmacokinetics, leading to increased tolerability, permitting higher dosing and resultant tissue drug levels.

Jung et al., 1999: studied enhanced solubility and dissolution rate of Itraconazole by a solid dispersion technique. The aim of the study was to improve the solubility and dissolution rate of a poorly water-soluble drug, Itraconazole, by a solid dispersion technique. Solid dispersion particles of Itraconazole were prepared with various pHindependent and dependent hydrophilic polymers and were characterized by differential scanning calorimetry, powder X-ray diffraction and scanning electron microscopy. Out of the polymers tested, pH-dependent hydrophilic polymers, AEA® and Eudragit® E 100, resulted in highest increases in drug solubility (range, 141.4–146.9-fold increases). The shape of the solid dispersion particles was spherical, with their internal diameter ranging from 1–10 mm. The dissolution rate of Itraconazole from the tablets prepared by spray drying (SD-T) was fast, with 90% released within 5 min. SD-T prepared with AEA® or Eudragit® E 100 at a 1:1 drug to hydrophilic polymer ratio (w:w) showed approximately 70-fold increases in the dissolution rate over a marketed product.

Yang et al., 2008: studied high bioavailability from nebulized Itraconazole nanoparticle dispersions with biocompatible stabilizers. A nebulized dispersion of

amorphous, high surface area, nanostructured aggregates of Itraconazole (ITZ):mannitol:lecithin (1:0.5:0.2, w/w) yielded improved bioavailability in mice. The ultra-rapid freezing (URF) technique used to produce the nanoparticles was found to molecularly disperse the ITZ with the excipients as a solid solution. Upon addition to water, ITZ formed a colloidal dispersion suitable for nebulization, which demonstrated optimal aerodynamic properties for deep lung delivery and high lung and systemic levels when dosed to mice. The ITZ nanoparticles produced supersaturation levels 27 times the crystalline solubility upon dissolution in simulated lung fluid. A dissolution/permeation model indicated that the absorption of 3μm ITZ particles is limited by the dissolution rate (BCS Class II behavior), while absorption is permeation-limited for more rapidly dissolving 230 nm particles. The predicted absorption half-life for 230 nm amorphous ITZ particles was only 15 min, as a result of the small particle size and high super-saturation, in general agreement with the *in vivo* results. Thus, bioavailability may be enhanced, by decreasing the particle size to accelerate dissolution and increasing permeation with an amorphous morphology to raise the drug solubility, and permeability enhancers.

Beule., 1996: studied pharmacology clinical experience and future development of Itraconazole. It is an orally active, broad-spectrum, triazole antifungal agent which has a higher affinity for fungal cytochrome P-450 than ketoconazole but a low affinity for mammalian cytochrome P-450. Itraconazole has a broader spectrum of activity than other azole antifungals and shows interesting pharmacokinetic features in terms of its tissue distribution. These properties have resulted in reduced treatment times for a number of diseases such as vaginal candidiasis, as well as effective oral treatment of several deep mycoses, including aspergillosis and candidiasis.

Hire et al., 2007: studied microparticulate drug delivery system for topical administration of Itraconazole. The microemulsion-based gels were evaluated for rheological behavior, in- vitro permeation studies and *in-vitro* antifungal activity. The invitro permeation studies was carried out on human cadaver skin, mounted on Keshary-Chien diffusion cell using 10% v/v methanolic solution of pH 1.2 phosphate buffer as diffusion medium and Candida albicans as a model fungus to evaluate the antifungal activity of Itraoconazole through the optimized formulations using cup plate method. Statistically significant increase in *in-vitro* permeation rate was found among the laboratory microemulsion based gel formulated when compared with conventional cream

formulation. The rheological behavior of the prepared systems showed pseudoplastic (shear thinning) flow pattern. The *in-vitro* antifungal activity of Itraconazole was found to be significant with microemulsion based gel. The microemulsion based gel is better choice of vehicle for delivery of Itraconazole as topical drug delivery system.

Miyake et al., 1999: studied characterization of Itraconazole/2-hydroxypropyl-β-cyclodextrin inclusion complex in aqueous propylene glycol solution. The interaction of Itraconazole, a triazole antifungal agent, with 2-hydroxypropyl-b-cyclodextrin (HP-β-CyD) in water and 10% v/v propylene glycol : water solution at pH 2.0 was investigated by the solubility method and ultraviolet and 1H-nuclear magnetic resonance (NMR) spectroscopies. The solubility of Itraconazole in water significantly increased as the concentrations of HP-β-CyD was augmented, showing an type phase solubility diagram. The upward curvature closely corresponded to the simulation curve which was calculated on the basis of the 1:2 (guest:host) complexation model. The 1:2 complex was formed even in the presence of 10% v:v propylene glycol, although the co-solvent system made the interaction with HP-β-CyD weaker due to the competitive inclusion. The ultraviolet spectroscopic studies also supported the 1:2 complex formation of Itraconazole with HP-β-CyD in 10% v/v propylene glycol:water solution at pH 2.0. The 1H-NMR spectroscopic studies suggested that the triazole and triazolone moieties of Itraconazole are involved in the 1:2 inclusion omplexation.

Kapsi et al., 2001: studied Processing factors in development of solid solution formulation of Itraconazole for enhancement of drug dissolution and bioavailability. This study investigated solid solutions of Itraconazole, a water insoluble antifungal, for improved dissolution and improved bioavailability. Influence of processing factors on drug and carrier properties in solid solution and subsequently on drug dissolution behavior was also studied. An optimized solid solution formulation was compared with marketed product in healthy human subjects under fasted and fed conditions for bioequivalency. Polyethylene glycol (PEG) and drug were made into a solid solution at 120 °C. The cooled, solid solution was then ground into granules of different sizes. Solid solutions of lower drug concentration dissolved at a faster rate, and drug dissolution improved considerably with increasing molecular weight of PEG. Initial treatment of Itraconazole with the wetting agent/cosolvent glycerol prior to making Itraconazole into a solid solution improved drug dissolution, and also reduced the PEG amount required to dissolve

drug to form solid solution. Addition of a polymer such as HPMC to the solid solution eliminated precipitation of drug following dissolution. As the granule size of the solid solution was reduced, precipitation of drug during dissolution became prominent.

Shao et al., 2007: studied recent advances and challenges in the treatment of invasive fungal infections. The frequency of invasive fungal infections (IFIs) has increased over the last decade with the rise in at-risk populations of patients. The morbidity and mortality of IFIs are high and management of these conditions is a great challenge. With the widespread adoption of antifungal prophylaxis, the epidemiology of invasive fungal pathogens has changed. Non-*albicans Candida*, non-*fumigatus Aspergillus* and moulds other than *Aspergillus* have become increasingly recognised causes of invasive diseases. These emerging fungi are characterised by resistance or lower susceptibility to standard antifungal agents. Invasive infections due to these previously rare fungi are therefore more difficult to treat. Recently developed antifungal agents provide the potential to improve management options and therapeutic outcomes of these infections. The availability of more potent and less toxic antifungal agents, such as second-generation triazoles and echinocandins, has led to considerable improvement in the treatment of IFIs. The authors reviewed the changing spectrum of invasive mycosis, the properties of recently developed antifungal agents and their role in the management of these infections.

Linn et al., 2012: studied Soluplus® as an effective absorption enhancer of poorly soluble drugs *in-vitro* and *in-vivo*. A novel solubility enhancing excipient (Soluplus®) was tested for its capability to improve intestinal drug absorption. BCS class II compounds danazol, fenofibrate and Itraconazole were tested both in vivo in beagle dogs and *in-vitro* in transport experiments across Caco-2 cell monolayers. *In-vitro* transport studies confirm the strong effect of Soluplus® on the absorption behavior of the three tested drugs. Furthermore, the increase of drug flux across Caco-2 monolayer is correlating to the increase in plasma AUC and Cmax in vivo. For these poorly soluble substances Soluplus® has a strong potential to improve oral bioavailability.

Patel and Agrawal., 2011: reviewed nanosuspension as an approach to enhance solubility of drugs. Nanosuspension consists of the pure poorly water-soluble drug without any matrix material suspended in dispersion. Preparation of nanosuspension is simple and applicable to all drugs which are water insoluble. A nanosuspension not only solves the

problems of poor solubility and bioavailability, but also alters the pharmacokinetics of drug and thus improves drug safety and efficacy.

Chudasama et al., 2011: investigated microemulsion system for transdermal delivery of Itraconazole. A new oil-in-water microemulsion-based (ME) gel containing 1% Itraconazole (ITZ) was developed for topical delivery. The solubility of ITZ in oils and surfactants was evaluated to identify potential excipients. The optimized microemulsion was incorporated into polymeric gels of Lutrol F127, Xanthan gum, and Carbopol 934 for convenient application and evaluated for pH, drug content, viscosity, and spreadability. *In-vitro* drug permeation of ME gels was determined across excised rat skins. These results indicate that the studied ME gel may be a promising vehicle for topical delivery of ITZ.

Hayes et al., 2011: studied fungal infection in heart-lung transplant recipients receiving single-agent prophylaxis with Itraconazole. An observational, retrospective study was performed to evaluate the rate of fungal infections in heart and lung transplant recipients at the University of Kentucky Medical Center over 4.5 years who received Itraconazole as a single therapy prophylaxis. Single-agent use with Itraconazole in heart or lung transplant recipients did not affect the rate of fungal infection as compared with previous reports. The incidence of fungal infection increased significantly within 3 months after escalation of immunosuppressant for treatment of acute rejection.

Lass Floral., 2011: reviewed triazole antifungal agents in invasive fungal infections. Triazole antifungals have emerged as front-line drugs for the treatment and prophylaxis of many systemic mycoses. Itraconazole also has a role in the treatment of fungal skin and nail infections as well as dematiaceous fungi and endemic mycoses. The therapeutic window for triazoles is narrow, and inattention to their pharmacokinetic properties can lead to drug levels too low for efficacy or too high for good tolerability or safety.

Das et al., 2011: studied Oral Itraconazole for the treatment of severe seborrhoeic dermatitis (SD). Itraconazole was given to 30 patients of SD in a dose of 100 mg twice daily for 1 week followed by 200 mg/day for first 2 days of the following 2 months. The response was noted on day 15, 30, 60, and 90. The clinical response was graded as markedly effective, effective, or ineffective. Clinical improvement (evaluated as markedly

effective or effective) was observed in 83.3% cases. The anti-inflammatory activity of oral Itraconazole suggests that it should be the first-line therapy in severe SD.

Wang and Huang., 2011: prepared Itraconazole-loaded liposomes coated by carboxymethyl chitosan and its pharmacokinetics and tissue distribution. This study described use of a film dispersion method to prepare Itraconazole-loaded liposomes (ITZ-Lips) prior to coating them with CMC. The concentrations of ITZ in selected organs were determined using reversed-phase high-performance liquid chromatography (HPLC). The biodistribution in mice was also changed after ITZ was encapsulated in CMC coated liposomes. CMC-ITZ-Lips performed significant lung targeting efficiency with AUC, The term has successfully encapsulated Itraconazole into carboxymethyl chitosan-modified liposomes for application of injection.

Buckner et al., 2011: measured posaconazole, Itraconazole, and hydroxyl Itraconazole in plasma/serum by high-performance liquid chromatography with fluorescence detection. A simple, sensitive high-performance liquid chromatographic method has been developed for the analysis of Itraconazole, hydroxyl - itraconazole, and posaconazole in serum/plasma. Few of the samples measured from patients participating in the clinical study attained concentrations of the drug/metabolite in serum that have been recommended for effective antifungal therapy.

Miller et al., 2012: studied flocculated amorphous Itraconazole nanoparticles for nhanced *in-vitro* supersaturation and in vivo bioavailability. Rapid flocculation of nanoparticle dispersions of a poorly water soluble drug, Itraconazole was utilized to produce amorphous powders with desirable dissolution properties for high bioavailability in rats. Antisolvent precipitation (AP) was utilized to form ITZ nanodispersions with high drug loadings stabilized with hydroxypropylmethylcellulose (HPMC) or the pH-sensitive Eudragit® L100-55 (EL10055). The HPMC dispersions were flocculated by desolvating the polymer through the addition of a divalent salt, and the enteric EL10055 by reducing the pH. The ability to generate and sustain high supersaturation in micellar media at pH 6.8 is beneficial for increasing bioavailability of ITZ by oral delivery.

Park et al., 2010: developed novel Itraconazole loaded solid dispersion without crystalline change with improved bioavailability. To develop a novel Itraconazole loaded solid dispersion without crystalline change with improved bioavailability, various Itraconazole-loaded solid dispersions were prepared with water, polyvinylpyrroline,

poloxamer and citric acid. The Itraconazole loaded solid dispersion at the weight ratio of Itraconazole/polyvinylpyrroline/poloxamer of 10/2/0.5 gave maximum drug solubility of about 20 mg/mL. It did not change the crystalline form of drug for at least 6 months, indicating that it was physically stable. It gave higher AUC, Cmax and tmax compared to Itraconazole powder and similar values to the commercial product, suggesting that it was bioequivalent to commercial product in rats. Thus, it would be useful to deliver a poorly water-soluble Itraconazole without crystalline change with improved bioavailability.

Li et al., 2010: developed and evaluated an Itraconazole loaded gelatine microcapsule with enhanced oral bioavailability. Various gelatine microcapsules were prepared using a spray-drying technique. Their physicochemical properties, dissolution, characteristics and pharmacokinetics in rats were evaluated and compared with those of a commercial product. The Itraconazole loaded gelatine microcapsule without ethanol developed using a spray drying technique at half the dose of the commercial product can deliver Itraconazole in a pattern that allows fast absorption in the initial phase, making it bioequivalent to the commercial product.

Engers et al., 2010: used a solid-state approach to enable early development compounds with selection and animal bioavailability studies of an Itraconazole amorphous solid dispersion. A solid-state approach to enable compounds in preclinical development is used by identifying an amorphous solid dispersion in a simple formulation to increase bioavailability. Solid dispersions were prepared with different at varied concentrations using two preparation methods (evaporation and freeze drying). The study demonstrated the utility of using an amorphous solid form with desirable physical properties to significantly improve bioavailability and provides a viable strategy for evaluating early drug candidates.

Chen et al., 2011: studied targeted brain delivery of Itraconazole via RVG29 anchored nanoparticles. 29-amino-acid peptide derived from rabies virus glycoprotein (RVG29) peptide conjugated Itraconazole-loaded albumin nanoparticles (RVG29-ITZNPs) was developed. . Cellular uptake of RVG29-ITZ-NPs was investigated by flow cytometry. Pharmacokinetics and brain distribution of RVG29-ITZ-NPs were investigated after intravenous administration of NPs. The results suggested that RVG29- ITZ-NPs can be exploited as a potential therapeutic formulation for the intracranial fungal infection

Tang et al., 2010: studied Pharmacokinetics and biodistribution of Itraconazole in rats and mice following intravenous administration in a novel liposome formulation. Novel Itraconazole (ITZ)-loaded liposomes (ITZ-LPs) were prepared and their pharmacokinetics and biodistribution were assessed in comparison with commercial formulations (ITZ-CD). The pharmacokinetics and biodistribution were studied in the rats and mice, and compared with commercially available formulations (Sporanox®) after administration by the tail vein at a dose of 10 mg/kg. In tissue distribution study, there were no differences of distributions in the lung between two formulations. Nevertheless, in the liver and spleen, Itraconazole levels for the group treated with ITZ-LPs were significantly higher than those for the group treated with ITZ-CD. Meanwhile, the low distribution of ITZ-LPs in heart and kidney was of great advantage to reduce the toxicity for heart and kidney. The results indicated that the ITZ-LPs can be a potential intravenous formulation of Itraconazole.

Jenoobi et al., 2010: studied effect of Itraconazole on the pharmacokinetics of diclofenac in beagle dogs. The objective of this study was to investigate the potential effect of Itraconazole on the pharmacokinetics of diclofenac potassium in beagle dogs after oral coadministration. Blood samples obtained for 8.0 hours post dose were analysed for diclofenac concentration using a validated high performance liquid chromatography (HPLC) assay method. The area under plasma concentration-time curve (AUC), maximum plasma concentration (Cmax), time to reach Cmax (tmax) and elimination half-life (t1/2), were calculated for diclofenac before and after Itraconazole administration. The coadministration of Itraconazole with diclofenac potassium has resulted in a significant reduction in AUC and Cmax of diclofenac, which was about 31 and 42%; respectively. No statistically significant differences were observed for tmax and t1/2 of diclofenac between the two phases. They concluded that oral coadministration of Itraconazole may have the potential to affect the absorption of diclofenac as indicated by the significant reduction in its AUC and Cmax in beagle dogs.

Al-Sarra et al., 2010: performed a comparative study of Itraconazole-cyclodextrin inclusion complex and its commercial product. Itraconazole (ITZ) solid complex using hydroxypropyl-beta-cyclodextrin (ITZ-HP-beta-CD) with 20% polyvinylpyrrolidone was prepared by a co-evaporation method. The complex improved antifungal activity against C. parapasilosis and C. albicans. Capsules containing ITZ-HP-beta-CD at a molar ratio of

1:3 with 20% polyvinylpyrrolidone have a faster dissolution rate than commercial capsules (Sporanox). The intraday precision showed a coefficient of variation less than 3.96%, and that for interday was less than 4.99%. The HPLC method was more accurate and precise than the antimicrobial and UV-spectrophotometric methods for determination of ITZ concentration present in the release medium.

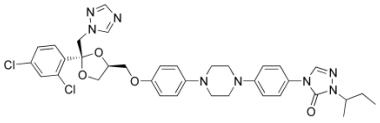
Tao et al., 2009: prepared and evaluated Itraconazole dihydrochloride for the solubility and dissolution rate enhancement. The morphology and mean size distribution study by SEM and DLS confirmed that the salt was dispersable nanoparticle aggregation. Aqueous solubility measurements showed that the solubility of the salt, its 1:1, 1:2 and 1:3 (w/w) physical mixtures with beta-cyclodextrin (beta-CD) was 6, 99, 236 and 388 times greater than Itraconazole. More than 94% of Itraconazole was dissolved out of the salt/beta-CD 1/3 physical mixture after 60min. The stability studies indicated that the physical mixture remained stable for 24 months in assay, the related substances and dissolution. They concluded that hydrochloride formation can significantly increase solubility and dissolution rate of Itraconazole, and the formulation of Itraconazole dihydrochloride/beta-CD (1/3) would be an environment-friendly, economic and practical alternative to the commercially available Itraconazole capsules (Sporanox).

Chudasama et al., 2011: investigated microemulsion system for transdermal delivery of Itraconazole. A new oil-in-water microemulsion-based (ME) gel containing 1% Itraconazole (ITZ) was developed for topical delivery. *In-vitro* drug permeation of ME gels was determined across excised rat skins. Furthermore, *in-vitro* antimycotic inhibitory activity of the gels was conducted using agar-cup method and Candida albicans as a test organism. The results indicated that the studied ME gel may be a promising vehicle for topical delivery of ITZ.

Drug Profile:-

Table 2.1

Drug profile of **Itraconazole**

Parameter	Description
Drug Name	Itraconazole
Chemical structure	
Molecular weight	705.64 g/mol
Chemical formula	$C_{35}H_{38}Cl_2N_8O_4$
IUPAC Name	1-(butan-2-yl)-4-{4-[4-(4-{[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]phenyl}-4,5-dihydro-1H-1,2,4-triazol-5-one
Appearance	White crystalline powder
Pharmacology	Treatment of fungal infections
Pharmacodynamics	Itraconazole is an imidazole/triazole type and highly selective inhibitor of fungal cytochrome P-450 sterol C-14 α-demethylation via the inhibition of the enzyme cytochrome P450 14α-demethylase. This enzyme converts lanosterol to ergosterol, and is required in fungal cell wall synthesis
Oral Absorption	About 55%
Protein binding	99.8%
Metabolism	Liver
Route of elimination	3-18% fecal excretion < 0.03% renal excretion About 40% in urine
Mean plasma half life	21 hours
Dosage forms	Tablets- 200 mg Capsules- 100 mg Suspension- 10 mg/ml, Parenteral- 10 mg/ml (250 mg for delivery of 200 mg)
Melting range	166.2 °C

CHAPTER 3

RESEARCH ENVISAGED AND PLAN OF WORK

3.1 Rationale

Itraconazole is an azole antifungal drug which is used and treat a variety of fungal infections. It has been extensively used as a second line agent, is broadly used clinically for patients because of infection by several microbial hosts treatment of fungal infections in normal and immunocompromised hosts, including Aspergillosis, Cryptococcus, Candida, Blastomyces, disseminated Penicillium mameffei infections, and Histoplasma capsulatum var. capsulatum (Arpan et al., 2011). The log P value of Itraconazole is 5.66 depicts the hydrophobicity of the drug. Oral and parenteral administration of the drug is associated with various side effects including headache, nausea, vomiting and abdominal pain. Further retinal, hepatic and renal toxicity were also observed in patients on high and prolonged dose of Itraconazole. Moreover, invasive parenteral delivery leads to poor patient compliance. Therefore, the present study is designed to develop the shampoo loaded with Itraconazole incorporated in SLNs to treat superficial fungal infection and to surpass the problems related to oral use.

3.2 Aim and objectives

3.2.1 Aim of work

The aim of the presented work is **"Formulation and Evaluation of Nano Particles of Itraconazole and Incorporation into Shampoo to Treat Scalp Infections"**.

3.2.2 Objectives

- To encapsulate hydrophobic drug "Itraconazole" into solid lipid nanoparticles and incorporation into the shampoo high drug payload for improved efficiency.
- To enhance the permeation and accumulation of drug in the scalp.
- Comparison of developed shampoo formulation with marketed antifungal shampoo.

3.3 Comprehensive plan of work

- Selection of excipients like lipid and stabilizer.
- Preformulation studies: compatibility study, solubility analysis, partition coefficient, prescreening studies for development of formulation.
- Development of SLNs containing drug by optimizing various chemical and physical variables by optimization technique.

RESEARCH ENVISAGED

- Physical and chemical characterization of nano particles with respect to entrapment efficiency, size analysis, transmission electron microscopy, *in vitro* drug release studies.
- Incorporation of SLNs into shampoo and characterization including pH, consistency, homogeneity, rheology, texture analysis, *in vitro* skin permeation studies and stability studies under stressed conditions developed.
- Antimicrobial studies of Itraconazole shampoo and comparison with marketed antifungal shampoo.

CHAPTER 4

MATERIALS AND METHODS

4.1 List of materials and equipment used during the stdy

Table 4.1

List of materials used in study

S.No.	Chemicals/Materials	Batch Number	Source/Manufacture
1	Itraconazole		
2	Cholesterol	SL55221302	Loba Chemie Pvt. Ltd. Mumbai, India
3	Span 60	L111061309	Loba Chemie Pvt. Ltd. Mumbai, India
4	Tween 80	L149771503	Loba Chemie Pvt. Ltd. Mumbai, India
5	Methanol	L206261609	Loba Chemie Pvt. Ltd. Mumbai, India
6	Lemon oil	14945	B.B Chemicals Pvt. Ltd.
7	Peppermint oil	LL11231207	Loba Chemie Pvt. Ltd. Mumbai, India
8	Sodium lauryl sulfate(SLS)	4411106	Titan Biotech Pvt. Ltd. Rajasthan, India
9	CMC	L1128773105	Loba Chemie Pvt. Ltd. Mumbai, India
10	EDTA	L118861403	Loba Chemie Pvt. Ltd. Mumbai, India
11	Sodium chloride	L186271601	Loba Chemie Pvt. Ltd. Mumbai, India
12	Potassium dihydrogen phosphate	LB276910	Loba Chemie Pvt. Ltd. Mumbai, India
13	Sodium acetate	SL34601201	Loba Chemie Pvt. Ltd. Mumbai, India
14	Glacial acetic acid	L117691401	Loba Chemie Pvt. Ltd. Mumbai, India
15	Disodium hydrogen phosphate	L117401402	Loba Chemie Pvt. Ltd. Mumbai, India
16	Chloroform	L218691701	Loba Chemie Pvt. Ltd. Mumbai, India

Table 4.2

List of equipment/software used in study

S.No.	Chemicals/Materials	Batch Number	Source/Manufacture
1	Bath sonicator		Raj Analytical Services, India
2	Electronic weighing balance	CY360	Shimadzu Co.Ltd., Japan
3	Eppendorf tubes	2 mL	Tarsons Products Pvt. Ltd. Kolkata, India
4	FTIR spectrometer	Spectrum 400	Shimadzu Co.Ltd. Japan
5	Hot air oven	Q-5247	Navyug, Mumbai, India
6	Heated/Magnetic stirrer	2 MLH	Remi, Pvt. Ltd. Mumbai, India
7	Membrane filters	0.22 µm, 0.45 µm	Advanced Micro Devices, Ambala Cantt, India
8	Particle size analyzer	Mastersizer 2000	Malvern Instruments Ltd. UK
9	pH meter	System 361	Systronic, µ pH system, India
10	UV spectrophotometer	2M9F36500	Shimadzu Co. Ltd. Japan
11	Transmission Electron Microscope (TEM)	H-7500	Hitachi Scientific Instruments, Tokyo, Japan
12	Optical microscope	10390	Kyowa, Gentner, Japan
13	Rheometer	(R heolab QC)	M/S Anton Paar, India
14	Autoclave	UEW- 501	Universal Engineering, Delhi, India
15	Laminar air flow bench	FED 2093	Microflow, India, Pvt. Ltd
16	Hot plate		Popular, India
17	Viscometer		Brookfield viscometer
18	Stability chamber	CHM 105	REMI Electrotechnik Pvt Ltd. vasai, Mumbai, India
19	Borosilicate Type- I glass	5 mL and 15 mL	Narang Scientific Works, Mumbai, India
20	Water bath shaker	NSW 133	Narang Scientific Works, Mumbai, India

S.No.	Chemicals/Materials	Batch Number	Source/Manufacture
21	Syringe and needle gauge no.21(Sterile)		
22	Franz Diffusion Cell Assembly		M/s Permegear, Inc., USA
23	Incubator	Universal 562	Narang Scientific Works Pvt Ltd. New Delhi, India
24	Software Design Expert®	Version 10.0.1	Licenced to StatEase, USA (www. Statease.com)

CHAPTER 5

EXPERIMENTAL WORK

5.1 Physicochemical characterization and identification of Itraconazole

5.1.1 Physical appearance test:

Itraconazole was categorised for dissimilar organoleptic properties such as color, odor and appearance. The results were compared with the COA provided by the manufacturer.

5.1.2 Melting point:

The melting point of Itraconazole was characterized by the capillary method (USP 30 NF 25, 2007). In this method, one end closed capillary tube is filled with the drug up to the height of 3mm and placed in the melting point apparatus. The thermometer is attached along to note the temperature. The temperature is record at which the drug starts to melt till the temperature where the whole drug gets melt.

5.1.3 Fourier transform infrared spectral analysis:

The FTIR spectrum of Itraconazole was observed by preparing potassium bromide pellets (Brammer *et al*., 1991). The finely ground Itraconazole powder was mixed with powdered potassium bromide and was pressed with a specific hydraulic compression. The prepared KBr pellet was then observed under Fourier transform infrared spectrometer (FTIR) and the spectrum was recorded. The FTIR spectrum obtained was compared with the spectrum obtained with Itraconazole standard.

5.2 Determination of absorbance maxima (λ_{max})

100 mg of itaconazole was accurately weighed by calibrated digital weighing balance and was dissolved in small quantity of methanol (porikh, s.k., *et al*., 2011). The solution was then transferred to 100 ml of volumetric flask. The volume was made up to 100 ml with methanol to obtain stock solution of 1mg/ml or 1000 µg/ml. One ml of stock solution (1000 µg/ml) was transferred to 10 ml volumetric flask and the volume was made up to the mark by adding methanol. Thus, the dilution with concentration 100 µg/ml was prepared. Half ml of stock solution (1000 µg/ml) was then farther transferred to 10 ml volumetric flask and the volume was made up by adding methanol to give results dilution of 50 µg/ml. Further, one ml of stock solution (100 µg/ml) was transferred to 10 ml volumetric flask and the volume was

made up by adding methanol for obtaining resulting dilution of 10 µg/ml. the prepared three dilutions were scanned on a double beam UV- visible spectrophotometer. The wavelength at which maximum absorbance was shown by both the dilutions, was recorded as absorbance maximum for Itraconazole.

5.3 Method validation for Itraconazole in methanol

5.3.1 Calibration Plot for Itraconazole in methanol

100 mg of Itraconazole was accurately weighed on calibrated digital weighing balance and was dissolved in small quantity of methanol (ICH, Q2 (R1) guidelines, 2005). The solution was then transferred to 100 ml of volumetric flask. The volume was made up to 100 ml to obtain stock solution of 1mg/ml or 1000 µg/ml. Further, one ml of stock solution (1000 µg/ml) was transferred to 10 ml volumetric flask and the volume was made up by adding methanol. Afterwards, again from the stock solution (100 µg/ml), 0.4, 0.8, 1.2, 1.6, 2 ml of solution was transferred into 10 ml volumetric flasks and volume was made up to 10 ml to form concentrations 4, 8, 12, 16, 20 µg/ml respectively. The absorbance was noted at value of obtained by scanning. The analysis was carried out in triplicate.

5.3.2 Linearity and Range

Linearity is the ability of the method to produce the results of experimental models that are directly proportional to analyte concentration within a given range (ICH, Q2 (R1) guidelines, 2005). Range is the interval between the upper and lower levels of analytes that can be determined with accuracy, precision and linearity. The accepted criteria for the linearity are that the correlation coefficient (R^2) should not be less than 0.990 for the least squares method of analysis of the line. Different aliquots from stock solution were sufficiently diluted to get solution in concentration ranging 4-20 µg/ml in triplicate. Calibration plots were obtained by plotting the graphs between absorbance versus concentration data and linear regression analysis was carried out for the same.

5.3.3 Accuracy

It represents the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found (ICH, Q2 (R1) guidelines, 2005). Accuracy was determined by performing recovery studies. It was performed by preparing different concentration levels (4, 12 and 20) µg/ml. The study was carried out in triplicate by preparing three sample solutions at each recovery level.

Absorbance was analyzed on a U.V spectrophotometer. Percentage mean recovery along with percentage R.S.D was calculated.

5.3.4 Precision

The accuracy of an analytical process expresses the familiarity of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (ICH, Q2 (R1) guidelines, 2005). The precision of proposed method was determined for three concentrations (4, 12 and 20 μg/ml) covering the entire linearity range by intraday (repeatability) and interday studies (intermediate precision). Intraday precision was determined by analyzing (4, 12 and 20 μg/ml) at three different time points on the same day and interday precision was determined by analyzing the solutions at three different time points on different days. For analyzing the precision, percentage R.S.D was calculated for intraday and interday precision studies.

5.3.5 Robustness

It is the measure of capacity to remain unaffected by small, but deliberate variations in the method parameters and provides an indication of its reliability during normal usage (ICH, Q2 (R1) guidelines, 2005). The robustness of proposed method was estimated by evaluating the interpersonal variation by recording % R.S.D.

5.3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detected is the lowermost amount of analyte in a sample which can be identified but not necessarily quantified as an exact value (ICH, Q2 (R1) guidelines, 2005). The limit of quantification of a separate analytical process is the lowermost amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy as shown in equation 5.1 and 5.2. Estimation of L.O.D and L.O.Q was based on the standard deviation of response and slope of the calibration curve.

$L.O.D = 3.3\ \sigma / S$ …….. (Equation 5.1)

(σ = Standard deviation of the intercept of linear regression equation)

(S= Slope of the regression equation)

$L.O.Q = 10\ \sigma / S$…… (Equation 5.2)

(σ = Standard deviation of the intercept of linear regression equation)

(S= Slope of the regression equation)

5.4 Preformulation studies

5.4.1.1 Drug excipient compatibility

Compatibility study was conducted for pure drug, excipients and drug: excipient combination in ratio of 1:1. The above mixtures were placed in glass containers and stored at temperature 50°C (ICH, Q1 A guidelines, 2005). The observation of combinations and pure drug and excipient sample was recorded 0^{th} and 15^{th} day physically for color change, appearance, state and lump formation.

5.4.1.2 Chemical characterization of drug excipients mixture

Chemical compatibility of drug excipients mixture was checked on 15th day by performing FTIR analysis of the drug with and without the excipients. The peaks of Itraconazole along with the excipients were observed. The effect of the excipients on the major peaks of Itraconazole was observed to find the compatibility of the drug and excipients.

5.4.2 Solubility studies

The solubility study of Itraconazole was carried out by using shake flask method with solvents like methanol, water, tween 80, pH 4.6 acetate buffer and pH 6.4 phosphate buffer (IP, 2007). Ten ml of each solvent was transfers, known additional quantity of drug was added to saturate the solution. The drug solutions were kept on water bath shaker by maintaining the temperature 25 ± 2^0C and by providing shaking of 80 horizontal strokes. The samples of drug solution in different solvents were taken and diluted suitably to observe the absorbance of drug by using U.V spectrophotometer at λ_{max} of Itraconazole. The drug concentration in each solvent was calculated from the standard plot and interpreted for calculation of solubility of drug in particular solvent.

5.4.3 Partition coefficient

The partition coefficient study was performed by using octanol and water. Both the solvents (5 ml) were filled in glass container to which 100 mg of drug was added (excess amount) (Florey, 2008). The mixture was allowed to shake for 24 hr at 37°C ± 2^oC. The solution was then transferred to the separating funnel and was shaken intermittently for one hour. The funnel was kept undisturbed to separate the two layers. The aqueous, organic layer were collected separately and the concentration of drug was found using U.V spectrophotometer.

5.5 Screening studies

5.5.1 Screening of the method for preparation of solid lipid nanoparticles

Solvent evaporation followed by homogenization method: Accurately weighed amount of Itraconazole, cholesterol and span 60 were transferred in to beaker and then transfer accurately measured quantity of methanol was added. The solubility was provided by applying sonication for 10-15 minutes. Measured quantity of tween 80 and water was added and kept for stirring on magnetic stirrer for 15min to produce an aqueous medium. The lipid phase was then slowly added into aqueous phase under continuous stirring and the organic solution was removed by evaporation. After removal of organic solvent, the nanoparticles are formed in aqueous medium. Finally, the aqueous medium was homogenized 1000-3000 rpm for 15-30 min.

5.6 Formulation Development trials

5.6.1 Preparation of optimized formulation by DoE technique

A Central composite design (*CCD*) was selected for two factors at three levels (X1 and X2) to optimize the response variables Y1 and Y2 respectively *i.e.* entrapment efficiency, drug permeation at one hour (Q1). Design expert software was used for employing this design. Table 5.2 summarizes an account of twenty eight experimental runs studied. Formulation at central point (0, 0) was studied in quintuplicate (Singh et al., 2006). Three levels -1, 0 and +1 were decided. On the basis of the preformulation studies, formulations were designed. CCD for two factors at three levels, each was selected to optimize the varied response. Design expert software was used for employing this design. The variables used were amount of Itraconazole, span 60 and methanol. The translation of coded factors levels and amount of ingredient is listed in table 5.1.

Table 5.1

Translation of experimental conditions into physical units

S.No.	Levels	Code factor	Itraconazole (mg)	Span 60 (mg)	Methanol (ml)
1	Low	-1	50	50	10
2	Medium	0	100	100	20
3	High	+1	150	150	30

5.7 Characterization and evaluation of nano suspension

5.7.1 Optical microscopy

Optical microscopy was done by optical microscope at 100 X using Oil immersion lens for viewing the abundance of nano particles and physical appearance (Raza *et al*., 2010). The morphological characteristics were studied for nanoparticle dispersion by optical microscopy. The photomicrographs of the preparations were obtained.

Table 5.2

Factor combination as per experimental design

Run no.	Drug (mg)	Span 60 (mg)	Methanol (ml)	Tween 80 (ml)	Homogenization (rpm)
F1	50	50	30	5	1000
F2	50	150	10	5	1000
F3	100	100	20	10	2000
F4	150	50	30	15	1000
F5	150	150	10	15	1000
F6	50	150	10	15	3000
F7	100	100	20	10	4000
F8	150	150	30	5	1000
F9	100	100	20	10	00
F10	200	100	20	10	2000
F11	100	100	20	00	2000
F12	100	100	00	10	2000
F13	100	100	20	10	2000
F14	50	150	30	15	1000
F15	50	150	30	5	3000
F16	150	50	10	5	1000
F17	150	50	30	5	3000
F18	50	50	10	5	3000
F19	100	100	40	10	2000
F20	100	100	20	20	2000
F21	100	200	20	10	2000
F22	150	50	10	15	3000
F23	100	0	20	10	2000
F24	150	150	30	15	3000
F25	150	150	10	5	3000
F26	50	50	30	15	3000
F27	50	50	10	15	1000
F28	0	100	20	10	2000

Name of contents	F1	F2	F3	F4	F5
Itraconazole(mg)	50	50	100	150	150
Cholesterol(mg)	200	200	200	200	200
Span 60(mg)	50	150	100	50	150
Methanol(ml)	30	10	20	30	10
Tween 80(ml)	5	5	10	15	15
Water(ml)	50	50	50	50	50
Homogenization(rpm)	1000	1000	2000	1000	1000

Fig 5.1 Representing F_1-F_5 formulas and their composition

Name of contents	F6	F7	F8	F9	F10
Itraconazole(mg)	50	100	150	100	200
Cholesterol(mg)	200	200	200	200	200
Span 60(mg)	150	100	150	100	100
Methanol(ml)	10	20	30	20	20
Tween 80(ml)	15	10	5	10	10
Water(ml)	50	50	50	50	50
Homogenization(rpm)	3000	4000	1000	0	2000

Fig 5.2 Representing F_6-F_{10} formulas and their composition

Name of contents	F11	F12	F13	F14	F15
Itraconazole(mg)	100	100	100	50	50
Cholesterol(mg)	200	200	200	200	200
Span 60(mg)	100	100	100	150	150
Methanol(ml)	20	00	20	30	30
Tween 80(ml)	00	10	10	15	5
Water(ml)	50	50	50	50	50
Homogenization(rpm)	2000	2000	2000	1000	3000

Fig 5.3 Representing F_{11}-F_{15} formulas and their composition

Name of contents (ml)	F16	F17	F18	F19	F20
Itraconazole (mg)	150	150	50	100	100
Cholesterol (mg)	200	200	200	200	200
Span 60 (mg)	50	50	50	100	100
Methanol (ml)	10	30	10	40	20
Tween 80 (ml)	5	5	5	10	20
Water (ml)	50	50	50	50	50
Homogenization (rpm)	1000	3000	3000	2000	2000

Fig 5.4 Representing F_{16}-F_{20} formulas and their composition

Name of contents (ml)	F21	F22	F23	F24
Itraconazole (mg)	100	150	100	150
Cholesterol (mg)	200	200	200	200
Span 60 (mg)	200	50	0	150
Methanol (ml)	20	10	20	30
Tween 80 (ml)	10	15	10	15
Water (ml)	50	50	50	50
Homogenization (rpm)	2000	3000	2000	3000

Fig 5.5 Representing F_{21}-F_{24} formulas and their composition

Name of contents (ml)	F25	F26	F27	F28
Itraconazole (mg)	150	50	50	0
Cholesterol (mg)	200	200	200	200
Span 60 (mg)	150	50	50	100
Methanol (ml)	10	30	10	20
Tween 80 (ml)	5	15	15	10
Water (ml)	50	50	50	50
Homogenization (rpm)	3000	3000	1000	2000

Fig 5.6 Representing F_{25}-F_{28} formulas and their composition

5.7.2 Transmission electron microscopy (TEM)

A drop of a sample was placed onto a carbon-coated grid and allowed to dry (Garg *et al*., 2007). The grid containing the sample was observed under the transmission electron microscope with an accelerating voltage of 120 kV. The nano particles were observed by focusing the lens. The images were then obtained after focusing the microscope with different magnifications of 5000-50000 X.

5.7.3 Particle size and size distribution analysis

Particle size was observed by Photon Correlation Spectroscopy (PCS) using Zeta sizer for the optimized nanoparticular formulation, which was prepared by solvent evaporation by homogenization (Esposito *et al*., 2006). The particle size and the size distribution were observed and were reported for the optimized formulation.

5.7.4 Drug entrapment efficiency

Itraconazole associated with nano suspension was separated from unentrapped drug using centrifugation method (Jia *et al*., 2004). SLNs were centrifuged at 20000 rpm for 30 min at controlled temperature. Supernatant containing unentrapped Itraconazole was withdrawn and measured by UV spectrophotometer at λ_{max} 260 nm against mixture of tween 80 and water. The amount of Itraconazole entrapped in SLNs was determined by calculating the entrapment efficiency as follows (equation 5.3):

$$EE\% = [At - Af/At \times 100] \ldots\ldots\ldots \quad \text{(equation 5.3)}$$

Where At is total amount of Itraconazole and Af is concentration of free Itraconazole. The entrapment efficiency was obtained by repeating the experiment in triplicate and the values were expressed as mean standard deviation.

5.7.5 *In vitro* drug release

Release of drug from the prepared nano suspension formulations was studied *in-vitro* using the semipermeable membrane dialysis bag. The dialysis bag was soaked in glycerin for whole night *i.e* for 12 h before performing the permeation study. The soaking helps to soften the membrane and opening of pores. One end of the tube was closed by tying a tight knot with a short piece of string about 1 cm from the end of the tubing. The tube was then filled the tube with 5 ml of prepared nano suspension through the open end. 100 mL of pH 5.5 phosphate buffer solution containing 30 % methanol was transferred into a clean 250-mL beaker. The dialysis bag was placed in a beaker containing the buffer solution and provided continues

stirring with the help of magnetic stirrer. The samples were collected from the beaker at different time intervals, by replacing the same amount of fresh sample to maintain the sink condition. The collected samples were analysed by using the UV Visible spectrophotometer at 260 nm for determining the concentration of drug.

5.8 Incorporation of prepared nano particles into shampoo

The shampoo prepared with variable ratio of components were as shown in table 5.3 by using the appropriate amount of prepared nano particles, peppermint oil, lemon oil, carboxy methyl cellulose (CMC) (2% gel) and sodium lauryl sulphate (SLS) the ingredients were transferred into motor pestle and mixed uniformly then it is transferred in clean and dried glass container. Small amount of EDTA was mixed in little amount of water and added in to the glass container. Then, few drops of saturated Nacl solution was added to modify or increase the viscosity of the shampoo. Finally, the volume was made up to the 100 ml by using the distilled water.

Table 5.3

Composition of shampoo with variable formulations

Formulation	SLNs (mg)	Lemon oil (ml)	Peppermint oil(ml)	SLS (mg)	CMC (ml)	EDTA	Saturated Nacl	Water
S1	500	4	3.5	30	7.5	2.5	qs	Qs
S2	500	6	4	37	10	3	qs	Qs
S3	500	3	5	45	8	2	qs	Qs
S4	500	5	3.5	55	9.5	4	qs	Qs
S5	500	6	4	60	9	3.5	qs	Qs
S6	500	4	5	65	8.5	4	qs	Qs

5.9 Evaluation of Itraconazole shampoo

5.9.1 pH determination

The pH of the shampoos (S_1-S_6) was determined by using digital pH meter.

5.9.2 Rheology

Viscosity of the shampoo preparations was determined by using Rheodyne Rheometer at 37 °C temperature with a shear rate (1/S) for 4 minutes. The plot of shear stress Vs shear strain was obtained. Similarly a plot of shear rate Vs viscosity was plotted. The equation was applied to power law Kt n. Where K represents the consistency, n helps to represents flow of the system.

5.9.3 Drug content and content uniformity

To ensure the drug content and uniform distribution of Itraconazole, 10 ml of prepared shampoo was taken and was shaken with sufficient quantity of methanol to extract the drug. The drug content was then determined by using UV spectrophotometer at λmax of 260 nm. The procedure was repeated in triplicate to ensure the uniform drug content. For analysis of uniform drug distribution the samples of shampoo were taken from 3 to 4 separate points and were determined spectrophotometrically.

5.9.4 In vitro permeation study

The *in vitro* permeation studies were carried by using the diffusion membrane, which was activated by keeping it in glycerine for overnight. The fresh diffusion membranes were taken each time for carrying *in vitro* permeation studies employing Franz diffusion cell (Mitkari *et al*., 2010). The different batches of shampoo were observed for their permeability to find the optimized batch. The diffusion membrane was mounted on the receptor chamber with cross sectional area of 3.91 cm^2. The receptor compartment was filled with 25 ml of pH 5.5 phosphate buffer. The cell was jacketed to maintain the temperature similar to skin *i.e*. 32 ± 0.5°C at 50 rpm. Each batch of shampoo was taken on the membrane and 5 ml aliquot of sample was withdrawn at different time intervals. Same amount of buffer was replenished to the compartment to maintain the phase equilibrium. The samples withdrawn were quantified spectrophotometrically at λ_{max} of 260 nm. Release study of blanks i.e. formulation without drug was also employed for each formulation (Fang *et al*., 2006; Qiu *et al*., 2008).

5.9.5 Stability study of shampoo

The stability study of shampoo was carried at different temperatures i.e. 4±3°C (Refrigerator; RF) and under stress conditions 50±2°C for a period of 15 days. The samples were taken periodically to analyze drug content for Itraconazole shampoo. A predetermined stability protocol was followed.

5.9.6 Analysis of Release Mechanism

In vitro release kinetics of Itraconazole from shampoo was analyzed by mathematical modeling. The *in vitro* drug release data obtained were fitted to various release kinetics models (Higuchi 1963; Korsemeyer, Gurny *et al.* 1983; Peppas and Sahlin 1989) *viz*., zero-order, first-order, Higuchi, Hixson-Crowell cube root and Korsemeyer-Peppas mathematical models. Selection of a suitable release model was based on values of r^2

(correlation coefficient), k (release constant) and n (diffusion exponent) obtained from curve fitting of release data. The data for optimized shampoo was compared with the marketed antifungal shampoo.

5.10 *In vitro* antifungal study

The prepared optimized shampoo formulation was tested in a triplicate manner using agar cup method against *Candida albican* strain. Cup of 10 mm in diameter were made aseptically in Sabouraud dextrose agar after being inoculated with tested fungal suspension strain by spreading on the agar surface. The cups were filled with formulation and control (Iraz® shampoo) by micro pipette . Then, the zone of inhibition of each cup was observed, the radius of the zone of inhibition was calculated and was compared with the control formulation. The observations were compared with the marketed antifungal shampoo as well.

CHAPTER 6

RESULTS AND DISCUSSION

6.1 Identification and characterization of Itraconazole

6.1.1 Physical description

The sample of Itraconazole was identified and characterized as per requirements of COA (certificate of analysis) issued by the manufacturer and (USP 30 NF 25, 2007). Results are shown in table 6.1.

Table 6.1

Identification and characterization of Itraconazole

Parameters	Specifications as per COA	Observation
Physical state	Solid	Solid
Colour	White	White
Odor	Odorless	Odorless

6.1.2 Melting point analysis

The observed experimental melting point by capillary method complies with the reported melting point as shown in table 6.2 (Brammer *et al*., 1991).

Table 6.2

Melting Point of Itraconazole

Parameter	Specification as per COA	Observation
Melting range	166.2^0	166^0-168^0

6.1.3 Identification of the drug Itraconazole by FTIR spectra

The FTIR spectra of the given sample showed comparable major absorption bands with that of reference standard of Itraconazole. The structure of Itraconazole is presented in Fig.6.1.The similarity in the characteristic peaks of obtained drug with that of reference standard confirmed the identity of the drug (Fig.6.2). The characteristic peaks represented the functional groups present along with the wave numbers associated with the structure.

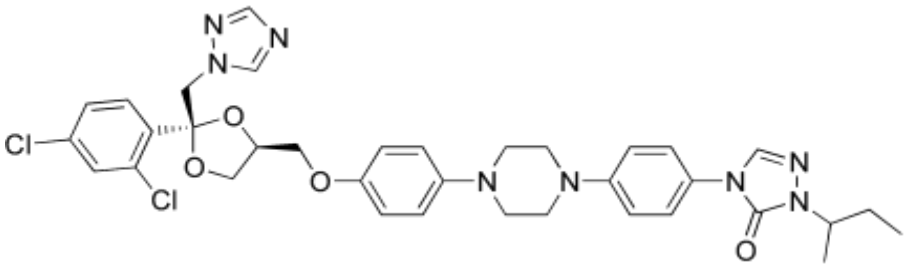


Fig. 6.1. Structure of Itraconazole (Yehia et al., 2008)

The FTIR spectrum of Itraconazole presented the characteristic peaks at 2821.95-3130.57 cm^{-1} due to –C-H- stretching vibrations. the stretching due to aromatic ring was absorbed by rare pick at wave number at 3066.92 cm^{-1}, –C-O stretching was characterized at 1224.84-1045.45 cm^{-1}, -C-N- stretch of amine group was observed at 1045.45-1330.93 cm^{-1}, peak at 1699.34 cm^{-1} was due to stretching of –C=O- and spectrum from 538.16-734.90 cm^{-1} indicating C-Cl stretching respectively.

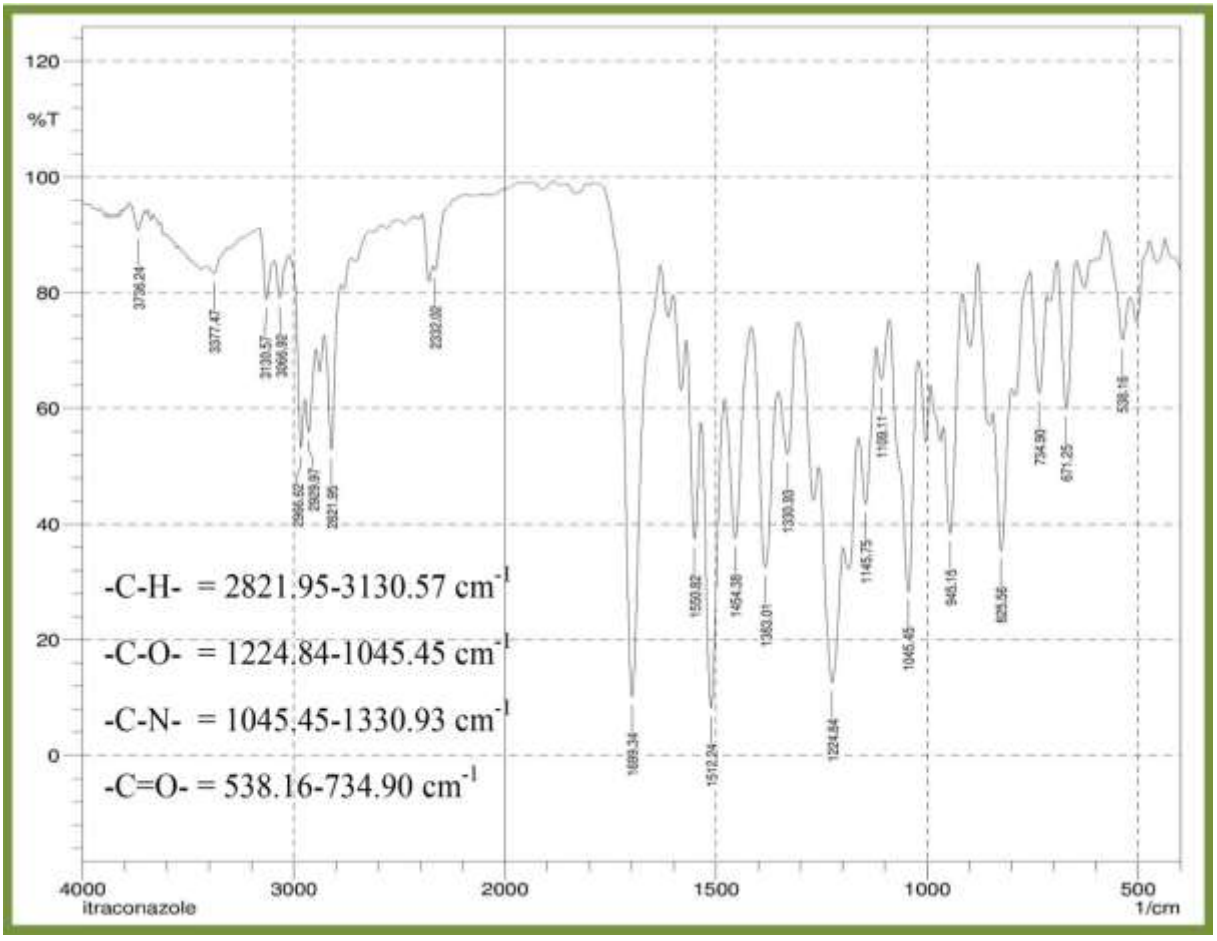


Fig. 6.2. FTIR Spectrum of Itraconazole on Shimadzu 1800S FTIR

6.2 Determination of absorption maxima (λ_{max}) of Itraconazole

The λ_{max} of Itraconazole was found to be 260 nm in methanol as solvent. The scanning of the drug was done in the range (200-400 nm) as shown in the Fig.6.3.

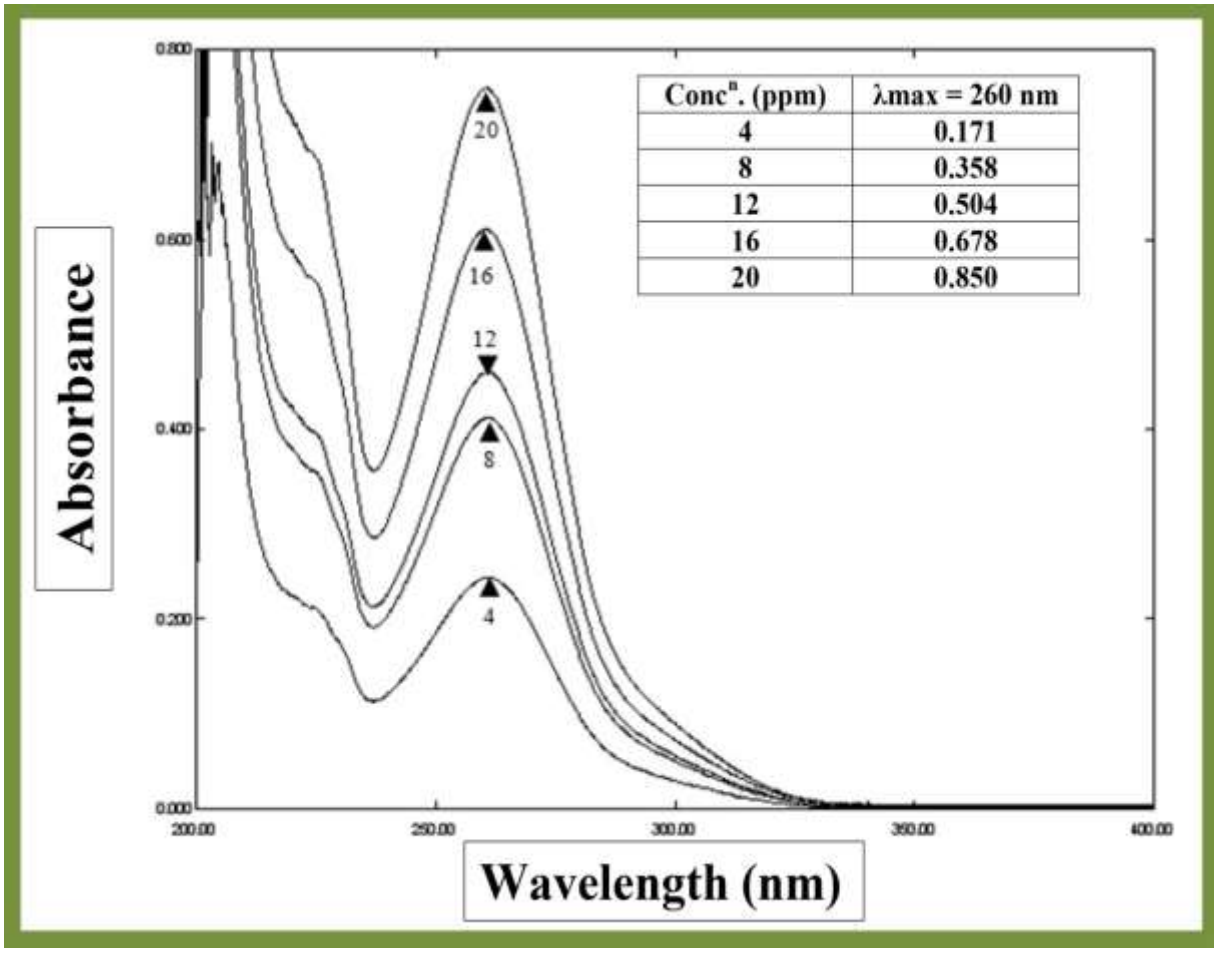


Fig. 6.3. Scan of Itraconazole in methanol when scanned between 200-400 nm

6.3 Analytical method validation of Itraconazole methanol

The U.V spectroscopic method was validated to check the suitability for the determination of drug (ICH, Q2 (R1) guidelines, 2005). The process of validation depicts whether the method is good for its intended purpose or not. The proposed method was validated according to ICH guidelines with respect to linearity, accuracy, precision, LOD, LOQ and robustness. The λ_{max} selected was 260 nm and the linearity was established in the range of 4-20 µg/ml with correlation coefficient, R^2= 0.9993. The validity of the proposed method was further assessed by recovery studies. The characteristic parameters are shown in table 6.8.

6.3.1 Calibration curve of Itraconazole in methanol

The calibration plot of Itraconazole was prepared by taking 4, 8, 12, 16, 20 µg/ml (table 6.3) concentrations of Itraconazole in methanol as shown in table 6.3. The experiments were performed in triplicate to find the standard deviation and percentage relative standard deviation. Absorbance range was found to be 0.171-0.850. The regression coefficient (R^2 value) was 0.9993 which showed linearity between 4-20 µg/ml concentrations. The Lambert Beer law was obeyed within the linearity range. The standard regression equation was found to be y = 0.0422 x + 0.0042.

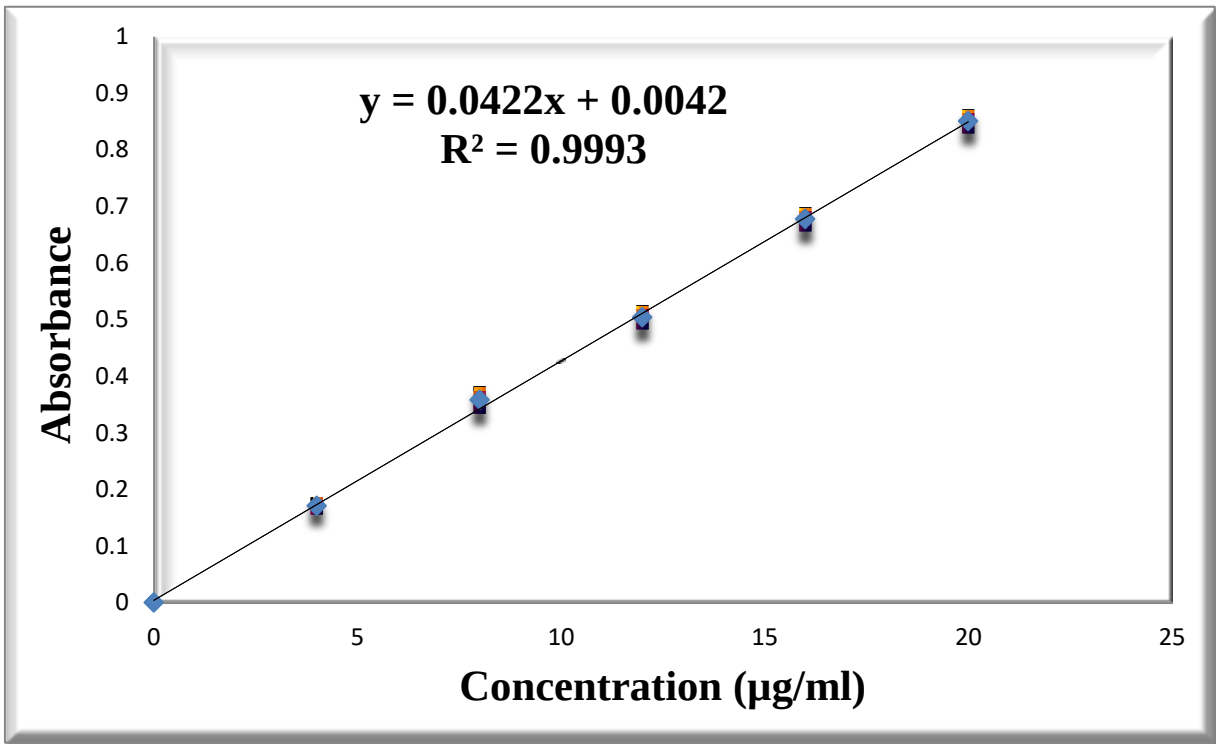


Fig. 6.4. Calibration curve of Itraconazole in methanol at 260 nm

Table 6.3

Absorbance of Itraconazole in methanol at 260 nm.

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Relative Standard deviation (RSD)
0	0	0
4	0.171 ± 0.002	1.456
8	0.358 ± 0.005	1.387
12	0.504 ± 0.004	0.892
16	0.677 ± 0.004	0.594
20	0.850 ± 0.004	0.508
Linear Regression (R^2)	**0.9993**	

6.3.2 Linearity and Range

Table 6.3 shows concentration and absorbance at 260 nm. Linearity was observed in range of 4-20 µg/ml at 260 nm with significant higher value of correlation coefficient, $r^2 = 0.999$ thus, follow Beer Lamberts law in this range as shown in Fig. 6.4.

6.3.3 Accuracy

Accuracy results as shown in table 6.4 displayed good reproducibility with RSD value below 2. The method was found to be accurate as percentage recovery was found to be within the range of 94.41-95.38 %. These results proved that the method is accurate for analysis of drug and can predict results related to experimental studies on drug.

Table 6.4

Result of accuracy study of Itraconazole in methanol.

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Mean recovery	% Relative Standard deviation (RSD)
4	3.777±0.049	94.41	1.30
12	11.413±0.049	95.11	0.43
20	19.077±0.042	95.38	0.22

6.3.4 Precision

The results of intraday, inter-day repeatability and reproducibility have been summarized in table 6.5 and 6.6 respectively. The results statistically were found to show good reproducibility with % RSD below 2. The results statistically were very close to the true value, confirmed by the S.D and R.S.D values. There was negligible variation in intraday and inter-day precision. Percentage recovery of intraday precision was between 97.03-99.42% and inter-day precision was between 97.83-99 %.

Table 6.5

Result of intraday precision study of Itraconazole in methanol.

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Mean recovery	% Relative Standard deviation (RSD)
04	3.977±0.037	99.42	0.93
12	11.643±0.009	97.03	0.08
20	19.427±0.071	97.13	0.37

Table 6.6

Result of inter-day precision study of Itraconazole in methanol.

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Mean recovery	% Relative Standard deviation (RSD)
04	3.913±0.062	97.83	1.59
12	11.787±0.039	98.22	0.33
20	19.800±0.245	99.00	1.24

6.3.5 Robustness

The results for robustness have been summarized in table 6.7 and were found to be within the variability limits. All the samples prepared by interpersonal variations and showed % RSD value below 2 and percentage mean recovery was also between 93.61-96.75 %. From the data it was found that slight changes by interpersonal do not affect the absorbance and method satisfactory. Therefore the technic and method can save appropriately for determination of drug sample.

Table 6.7

Result of robustness of Itraconazole by interpersonal variations

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Mean recovery	% Relative Standard deviation (RSD)
4	3.870±0.037	96.75	0.97
12	11.233±0.038	93.61	0.34
20	19.011±0.048	95.05	0.26

6.3.6 Limit of Detection and Limit of Quantification

The LOD and LOQ for the drug were found to be 0.213 µg/ml and 2.085 µg/ml respectively as given in table 6.8. These results demonstrate that the method is sensitive and can detect the drug in the above concentration range with linearity.

6.4 Preformulation studies

6.4.1 Drug excipients compatibility

The results obtained from compatibility study of Itraconazole with various excipients showed no physical and chemical incompatibility between Itraconazole and excipients under stress conditions. However, overall observations depicted no change in colour, appearance, lumps and state of either drug, excipient alone or in combination. The compatibility was

assured by carrying out FTIR spectral analysis of drug with excipients and by comparing the peaks (Fig 6.5 and 6.6) of mixture with that of drug and individual excipients.

Table 6.8

Characteristics for Itraconazole in methanol

Parameters	Values
λmax (nm)	260
Linearity range (µg/ml)	4-20
Slope	0.0422
Intercept	0.0042
Correlation coefficient (R^2)	0.9993
Accuracy (Percentage mean recovery)	94.41-95.38
Intraday Precision (Percentage mean recovery)	97.03-99.42
Interday Precision (Percentage mean recovery)	97.83-99.00
Robustness (Percentage mean recovery)	93.61-96.75
LOD (µg/ml)	0.213
LOQ (µg/ml)	2.085

Table 6.9

Drug and excipients in 1:1 ratio for compatibility studies

S.No.	Ingredients	Color	Appearance	State	Lumps
1	Itraconazole	White	Crystalline	Solid	Not present
2	Chloroform	White	Crystalline	Solid	Not present
3	Span 60	White	Amorphous	Solid	Not present
4	Tween 80	Yellow	Crystalline	liquid	Not present
4	Itraconazole : Chloroform	White	Crystalline	Solid	Not present
5	Itraconazole : Span 60	White	Amorphous	Solid	Not present
6	Itraconazole : Chloroform : Tween 80		Crystalline		Not present
7	Itraconazole : Chloroform : Span 60 : Tween 80	White	Crystalline	Solid	Not present

Table 6.10

Drug and excipients in 1:1 ratio at different time intervals

Ingredients	1st Day	2nd Day	3rd Day	10th Day	15th Day
Itraconazole					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Chloroform					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Span 60					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Tween 80					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Itraconazole : Chloroform					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Tween 80					
Itraconazole : Span 60					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Itraconazole : Tween 80					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√

Lumps	√	√	√	√	√
Itraconazole : Chloroform : Span 60 : Tween 80					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Where √ represents same as initial (no change), × represents changes observed					

6.4.2 Solubility analysis of Itraconazole

The solubility data was obtained for Itraconazole at 25°C using an ultraviolet absorption assay method to determine the concentration of drug present in the saturated solutions (IP, 2010). The solubility profile of drug with the organic solvents and buffers was helpful to determine that whether the drug was dispersed or solublized in the organic solvents and buffers systems. The solubility profile in the decreasing order of solubility was found to be as follows: chloroform > methanol > Tween 80 > pH 5.5 phosphate buffer > pH 4.6 acetate buffer > water. The pH solubility profile of Itraconazole was generated and was reported and shown in table 6.11. The solubility profile signifies that the drug get sparingly solublized in the methanol so it can be dispersed in the lipid system during the formulation. The dispersibility can help in enhancing the pay load of drug in the SLNs. Thus, the solubility profile helped to generate the supportive information regarding the final formulation.

6.4.3 Partition coefficient of Itraconazole

The partition coefficient of Itraconazole between octanol and water (log P) was determined (Florey, 2008). The study indicated that Itraconazole has a log P value equals to 5.73. *n*-octanol and pH 8.1 aqueous buffer solution. As the drug was poorly water soluble. Therefore, to known correlation of drug in aqueous phase pH 8.1 phosphate buffer was used.

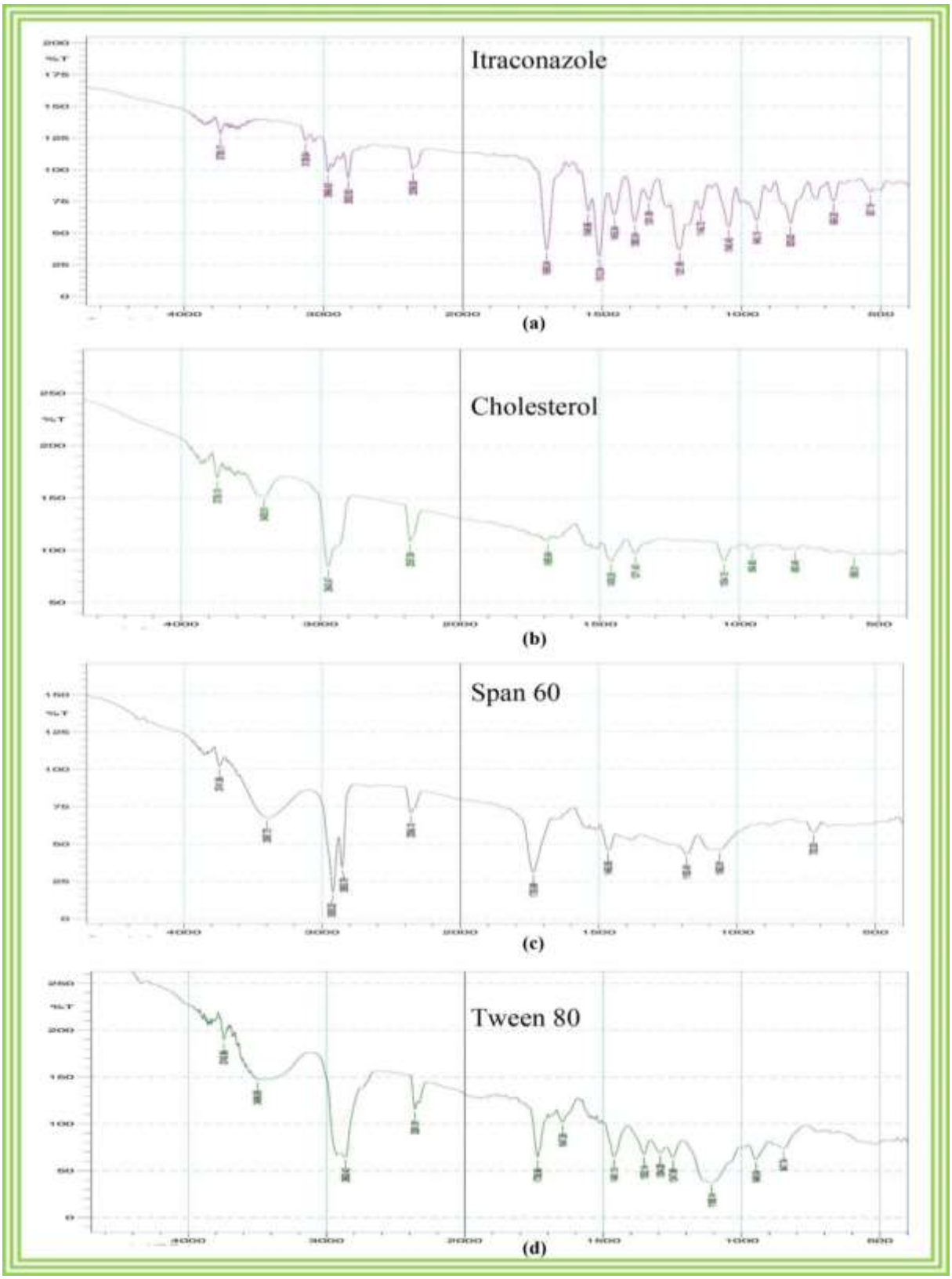


Fig. 6.5. FTIR spectra of drug and excipients (a) Itraconazole, (b) Cholesterol, (c) Span 60, (d) Tween 80

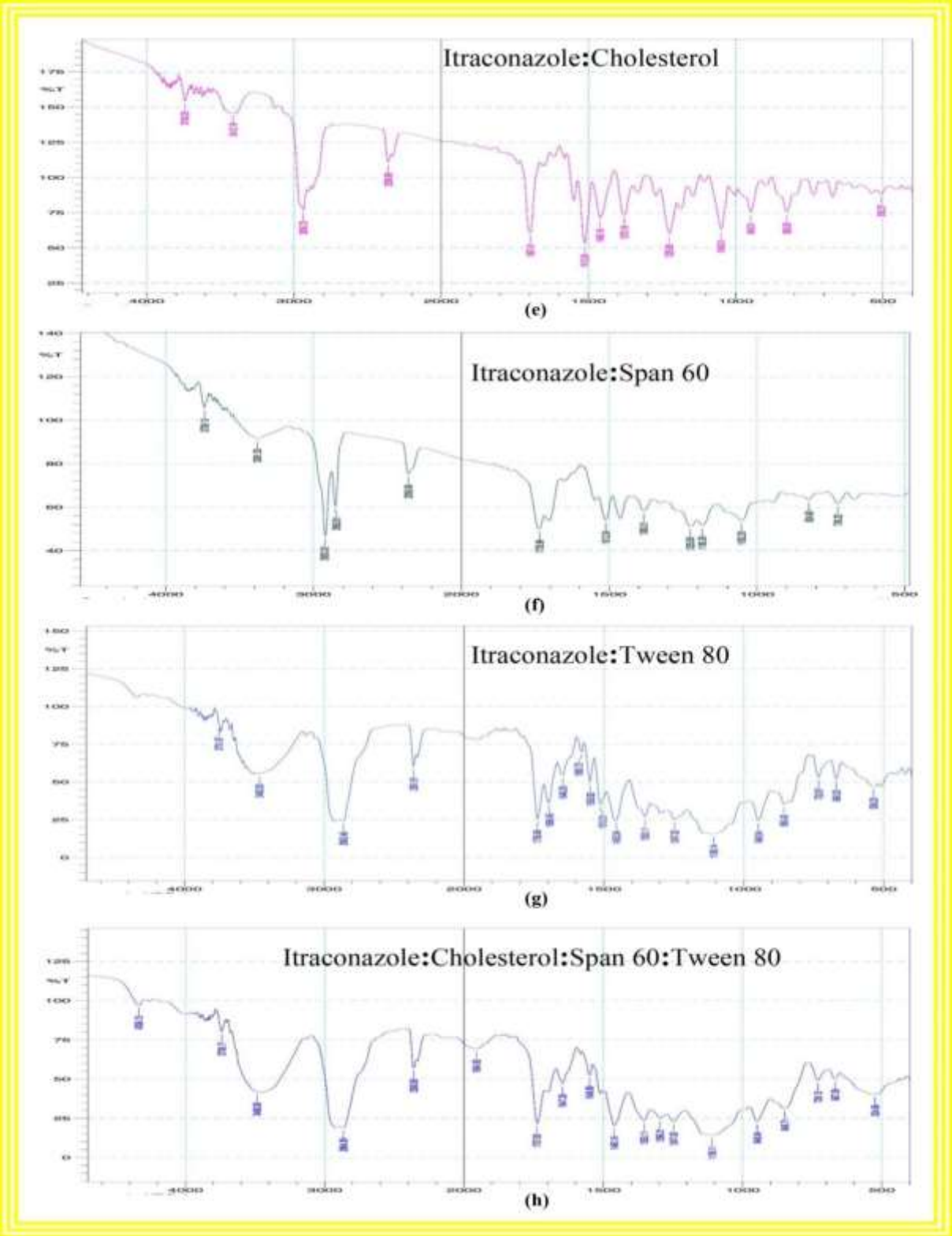


Fig. 6.6. FTIR spectra of drug and excipients (e) Itraconazole: Cholesterol, (f) Itraconazole: Span 60, (g) Itraconazole: Tween 80 (h) Itraconazole: Cholesterol: Span 60: Tween 80.

Table 6.11

Solubility of Itraconazole in various organic solvents and buffers IP, 2010)

S.No.	Solvent (variable pH)	Solubility (mg/ml)	Solubility Profile
1.	Chloroform	50	Soluble
2.	Methanol	20	Sparingly soluble
3.	Tween 80	5.31	Slightly soluble
4.	pH 5.5 phosphate buffer	1.63	Slightly soluble
5.	pH 4.6 phosphate buffer	0.013	Insoluble
6.	Water	0.0003	Insoluble

6.4.4 Pre-screening study for selection of ratio of components

Pre-screening study was done to select the levels for design of experiment. For this, the formulations were prepared with variable amount of Itraconazole (Drug). The purpose was to incorporate maximum amount of drug without affecting the characteristic features of nano suspension related to particle size and clarity (Shahul *et al*., 2014). On the basis of literature review, the ratios of components like lipid, surfactant and solvent were selected and kept constant. In order to finalize the amount of drug, various formulations were taken in to consideration in further optimization. Where optimize, the level of independent variables were varied for low, medium and high and trials were correlated as maintained in further section. After the pre-screening studies, it was found that by varying the formula for components the drug con incorporated in them, the range of 25-200 mg and solvent evaporation by high pressure homogenization was the best suitable method to produce nano suspension with smaller particle size (expected within nano range). The ratio of the components were screened by optical microscopy as shown in table 6.12 and Fig.6.7.

Table 6.12

Screening the ratio of components for formulation

Batch No.	Itraconazole(mg) : Methanol(ml)	Spherical particles	Maximum size (µm)
T1	25 : 20	Less abundant	36
T2	50 : 20	Present	22.1
T3	75 : 20	Less abundant	32.8

T4	100 : 20	Less abundant	38.2
T5	125 : 20	Present	21.4
T6	150 : 20	Present	14.1
T7	175 : 20	Absent	-
T8	200 : 20	Absent	-

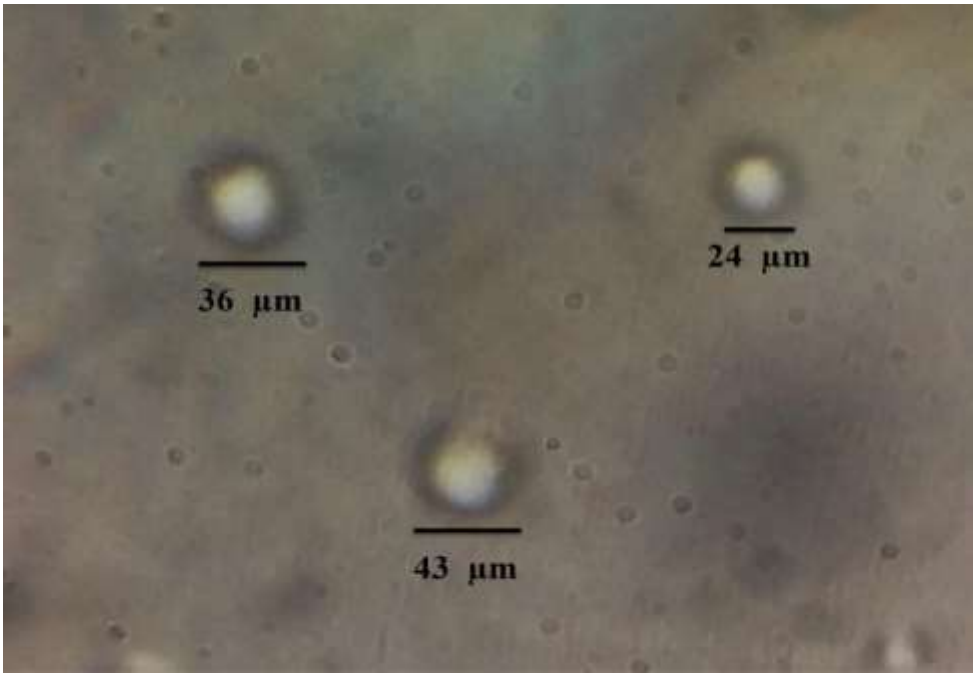


Fig. 6.7. Representative optical photomicrographs of SLNs formulation (T6)

6.5 Formulation Development trials

6.5.1 Optimization of nano suspension by central composite design

The design of optimization contained five independent variables (A, B, C, D, E) and two dependent variables (Y1 and Y2). The independent variables were drug (mg), span 60 (mg), methanol (ml), tween 80 (ml) and homogenization (rpm) respectively, whereas, the Y variables were percentage entrapment efficiency and percentage drug permeability. According to the design, 28 formulations were suggested. Each of them were formulated and analysed for two different responses. The results were analyzed by using polynomial modelling approach using the software, design expert. Various statistical parameters, ANOVA were applied and the responses were evaluated as shown in table 6.13.

Table 6.13

Factor combination and responses as per *central composite design*

Run no.	Drug (mg)	Span 60 (mg)	Methanol (ml)	Tween 80 (ml)	Homogeni-zation (rpm)	Entrapment efficiency (%)	Drug permeability (%)
F1	50	50	30	5	1000	80.14	79.07
F2	50	150	10	5	1000	76.82	69.80
F3	100	100	20	10	2000	60.58	56.08
F4	150	50	30	15	1000	91.48	89.90
F5	150	150	10	15	1000	57.42	41.62
F6	50	150	10	15	3000	76.68	62.09
F7	100	100	20	10	4000	57.98	44.06
F8	150	150	30	5	1000	42.89	31.45
F9	100	100	20	10	00	56.64	43.17
F10	200	100	20	10	2000	14.45	58.80
F11	100	100	20	00	2000	55.85	56.10
F12	100	100	00	10	2000	00.00	00.00
F13	100	100	20	10	2000	53.24	66.63
F14	50	150	30	15	1000	87.46	88.28
F15	50	150	30	5	3000	77.58	75.74
F16	150	50	10	5	1000	53.48	44.54
F17	150	50	30	5	3000	56.32	68.75
F18	50	50	10	5	3000	78.10	72.24
F19	100	100	40	10	2000	61.37	41.18
F20	100	100	20	20	2000	51.66	30.77
F21	100	200	20	10	2000	53.48	32.50
F22	150	50	10	15	3000	56.79	66.90
F23	100	0	20	10	2000	29.86	24.52
F24	150	150	30	15	3000	51.74	44.55
F25	150	150	10	5	3000	45.58	33.08

F26	50	50	30	15	3000	76.97	64.79
F27	50	50	10	15	1000	77.91	80.58
F28	0	100	20	10	2000	00.00	00.00

Table 6.14

Physical appearance of suggested formulations by (DOE)

Run no.	Clarity	Turbidity	Settling	Re-dispersion	uniformity
F1	4	2	✓	✓	✓
F2	3	3	✓	✓	✓
F3	2	4	✓	✓	✓
F4	5	1	×	-	✓
F5	1	5	✓	×	✓
F6	2	4	✓	✓	✓
F7	1	5	✓	✓	✓
F8	3	3	✓	×	✓
F9	3	3	✓	✓	✓
F10	2	4	✓	✓	✓
F11	2	4	✓	✓	✓
F12	1	5	✓	×	✓
F13	2	4	✓	×	✓
F14	5	1	×	-	✓
F15	4	2	✓	✓	✓
F16	2	4	×	-	✓
F17	4	2	✓	✓	✓
F18	1	5	✓	×	✓
F19	3	3	✓	✓	✓
F20	4	2	✓	✓	✓
F21	2	4	✓	×	✓
F22	3	3	✓	✓	✓
F23	2	4	✓	×	✓
F24	3	3	✓	✓	✓
F25	1	5	✓	×	✓
F26	3	3	✓	✓	✓
F27	5	1	×	-	✓
F28	5	1	✓	×	✓

1 = 0-20%, 2 = 20-40 3 = 40-60%, 4 = 60-80%, 5 = 80-100%

✓ = Yes, × = No, - = Transparent

6.6 characterization and evaluation of nano suspension

6.6.1 Optical microscopy

The prepared formulations were examined for optical microscopy as shown in Fig.6.7. Optical microscopy showed that the nano particles were observed in formulations studied at 100 X. The micrographs of nano particles revealed the presence of vesicles which were nano in appearance as shown in Fig.6.8.

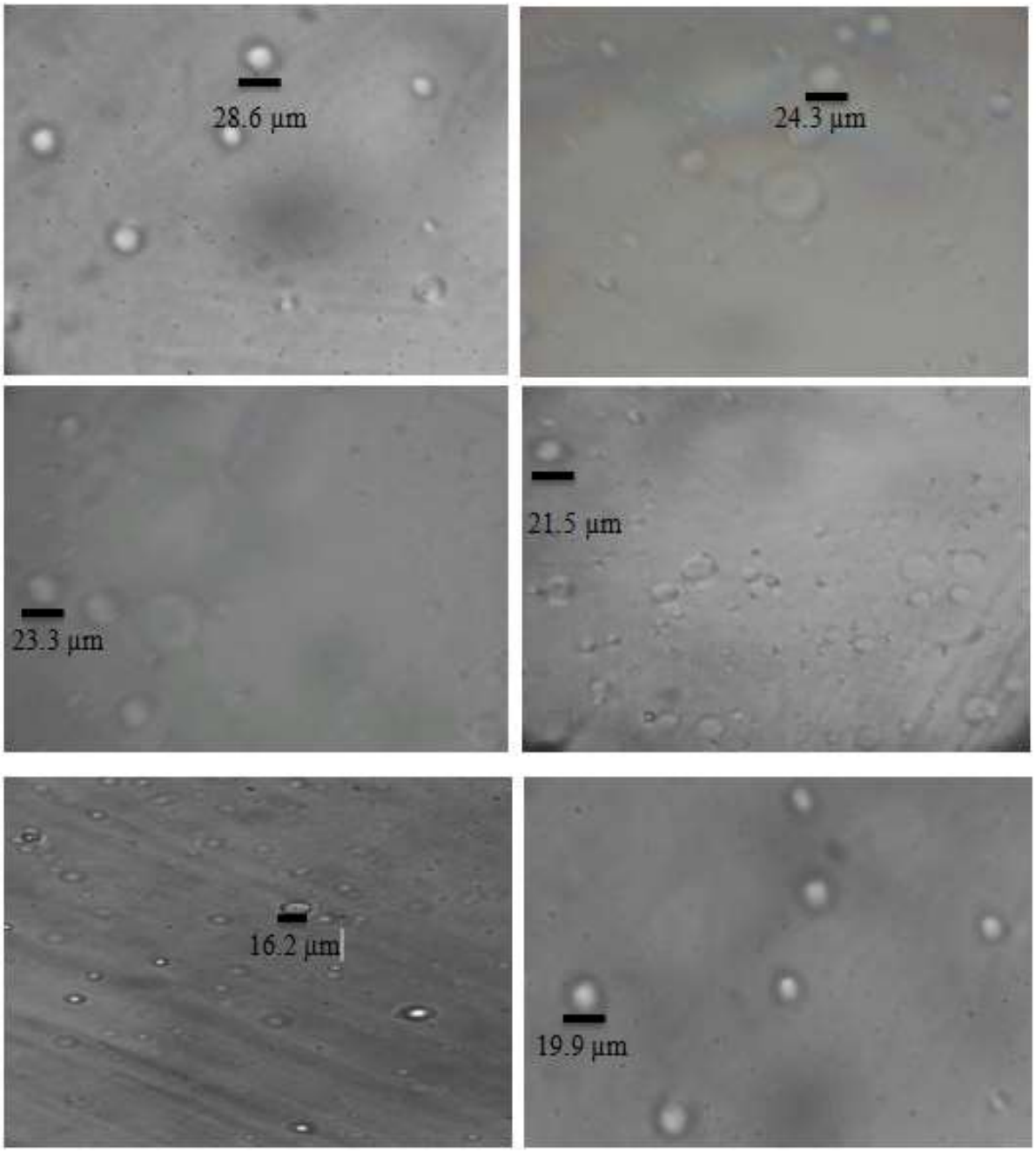


Fig. 6.8. Optical photomicrographs of some representative nano suspension formulations

6.6.2 Transmission electron microscopy (TEM)

TEM photomicrographs of some representable nano suspension dispersion are shown in Fig 6.9-6.12. The grid containing the sample was observed under the transmission electron microscope with an accelerating voltage of 120 kV with magnification between 5000 X – 50000 X. nano particles were discrete, uniform and spherical. The diameter was found to be within the range of 10-150 nm.

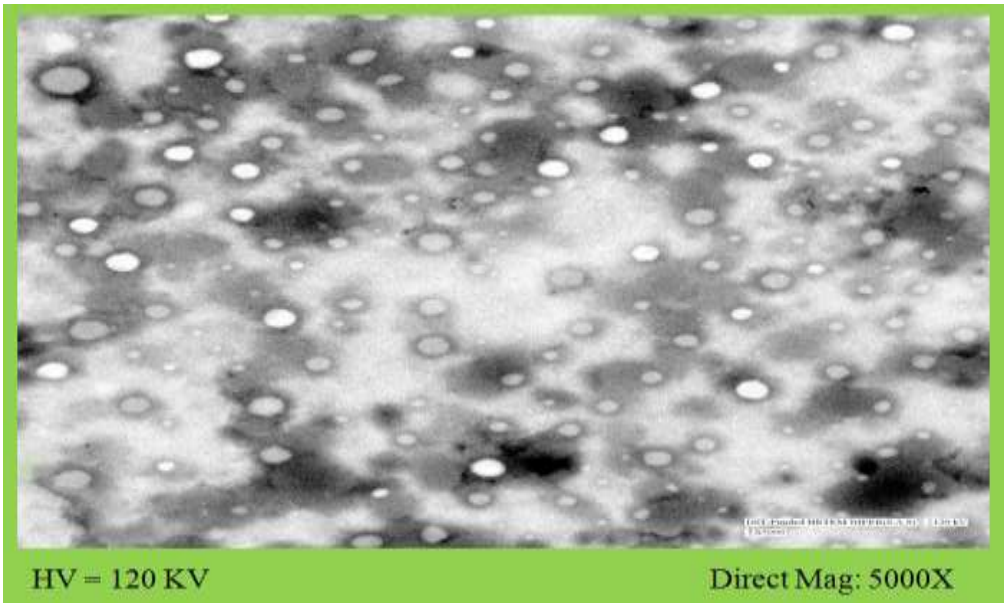


Fig. 6.9. Transmission electron micrograph of (F4) nano suspension dispersion with magnification of 5000 X

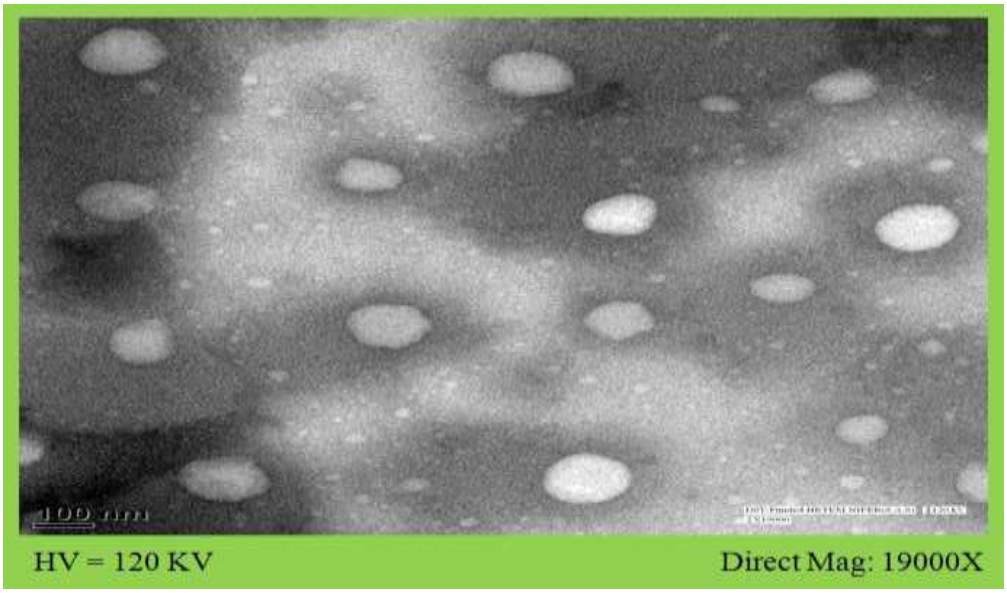


Fig. 6.10. Transmission electron micrograph of (F4) nano suspension dispersion with magnification of 19000 X

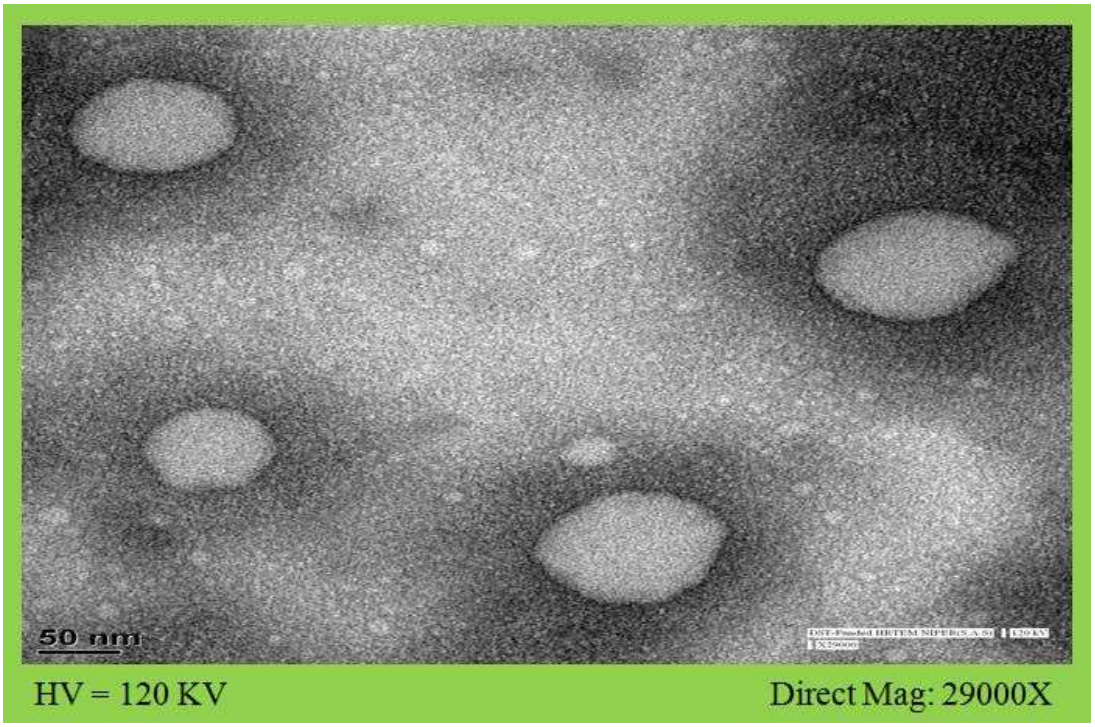


Fig. 6.11. Transmission electron micrograph of (F4) nano suspension dispersion with magnification of 29000 X

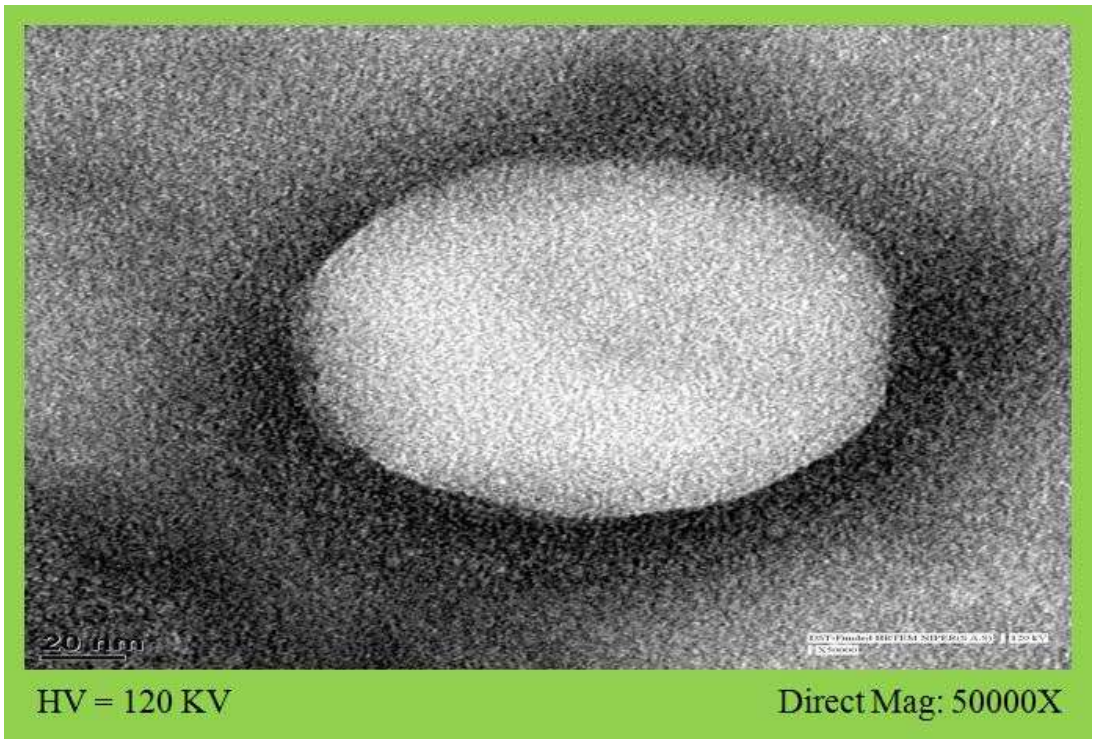


Fig. 6.12. Transmission electron micrograph of (F4) nano suspension dispersion with magnification of 50000 X

6.6.3 Particle size analysis

The mean particle size and Polydispersity Index (PI) of nano particles are presented in table 6.15. The differences in the particle size of SLNs formulations prepared with variable ratios of drug, span 60, methanol, tween 80 and homogenization were utilized to find the optimized formulation. The particle sizes were falling in the range of 10 - 100 nm as shown in Fig. 6.13. In general, nano and microcarriers with Polydispersity Index (PI) value higher than 0.3 shows large size distributions and have the tendency to aggregate (Junyaprasert *et al.*, 2008). Smaller value of PI (PI<0.3) indicates a homogeneous population of nano particles (Maurya *et al*., 2011). The optimized formulation showed average particle size of 94.5 nm with PI of 0.261 as shown in Fig. 6.14. This shows that the optimized nano suspension formulation is homogeneous with uniform distribution.

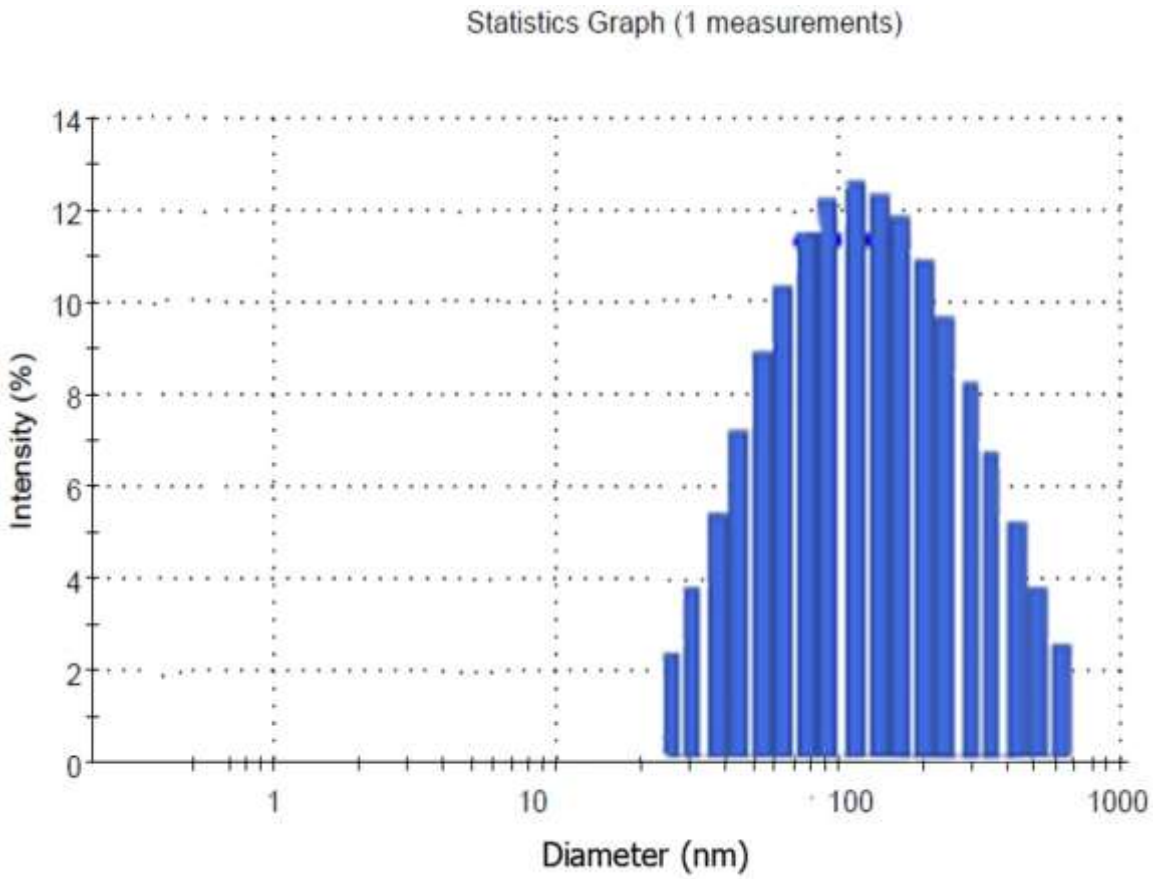


Fig. 6.13. Size distribution of optimized SLNs formulation (F4)

Table 6.15

Cumulate results for the particle size distribution

Cumulate Results	
Diameter (d)	90.7 nm
Polydispersity Index (PI)	0.261
Diffusion Const. (D)	8.349e-009 cm^2/sec
Measurement Condition	
Temperature	25.1 ^{0}C
Diluent Name	Water
Refractive Index	1.3328
Viscosity	0.8858 (cP)
Scattering Intensity	4360 (cps)

6.6.4 Entrapment efficiency

Table 6.16 represents the data for entrapment efficiency. Fig. 6.14 comparatively depicts entrapment efficiency of all the 28 formulations. From the entrapment data it was observed that the ratio of the five components within an optimum range offered good entrapment efficiency (Rai *et al*., 2013). The effect of the quantity of methanol was also determined from the study. When the formulation with absence of methanol was prepared, it appeared as a turbid form and was not able to form the nano suspension dispersion. The entrapment efficiency could not be determined because of the turbidity. Thus, it was observed that the methanol plays an important role in the formation of the nano particles by promoting particle dispensability. Thus, it may be inferred that as the nano suspension shows better drug entrapment, they also have effect on the drug loading (Guo et al., 2010; Phan *et al*., 2011). The enhanced entrapment efficiency is because of the structure of the composition of nano suspension which offers more space for the drug particles to get entrapped. The maximum entrapment efficiency was absorbed for F_4 with 91.48 % and minimum was for F_{10} with 14.45 %, although formulation F_{12} and F_{28} offered no entrapment due to absence of methanol and drug in the formula respectively.

Table 6.16

Entrapment efficiency of nano particles of prepared formulations

Run. no.	Formulations	Free drug content (mg)	Total drug content (mg)	Entrapment efficiency (%)
1	F_1	9.93	50	80.14
2	F_2	11.59	50	76.82
3	F_3	59.12	100	60.58
4	F_4	12.77	150	91.48
5	F_5	63.86	150	57.42
6	F_6	11.66	50	76.68
7	F_7	63.03	100	57.98
8	F_8	85.66	150	42.89
9	F_9	65.05	100	56.64
10	F_{10}	171.09	200	14.45
11	F_{11}	66.23	100	55.85
12	F_{12}	00.00	00	00.00
13	F_{13}	70.14	100	53.24
14	F_{14}	6.27	50	87.46
15	F_{15}	11.21	50	77.58
16	F_{16}	69.79	150	53.48
17	F_{17}	65.52	150	56.32
18	F_{18}	10.95	50	78.10
19	F_{19}	57.94	100	61.37
20	F_{20}	72.51	100	51.66
21	F_{21}	69.79	100	53.48
22	F_{22}	64.81	150	56.79
23	F_{23}	105.21	100	29.86
24	F_{24}	72.39	150	51.74
25	F_{25}	81.64	150	45.58
26	F_{26}	11.52	50	76.97

27	**F_{27}**	11.04	50	77.91
28	**F_{28}**	00.00	00	00.00

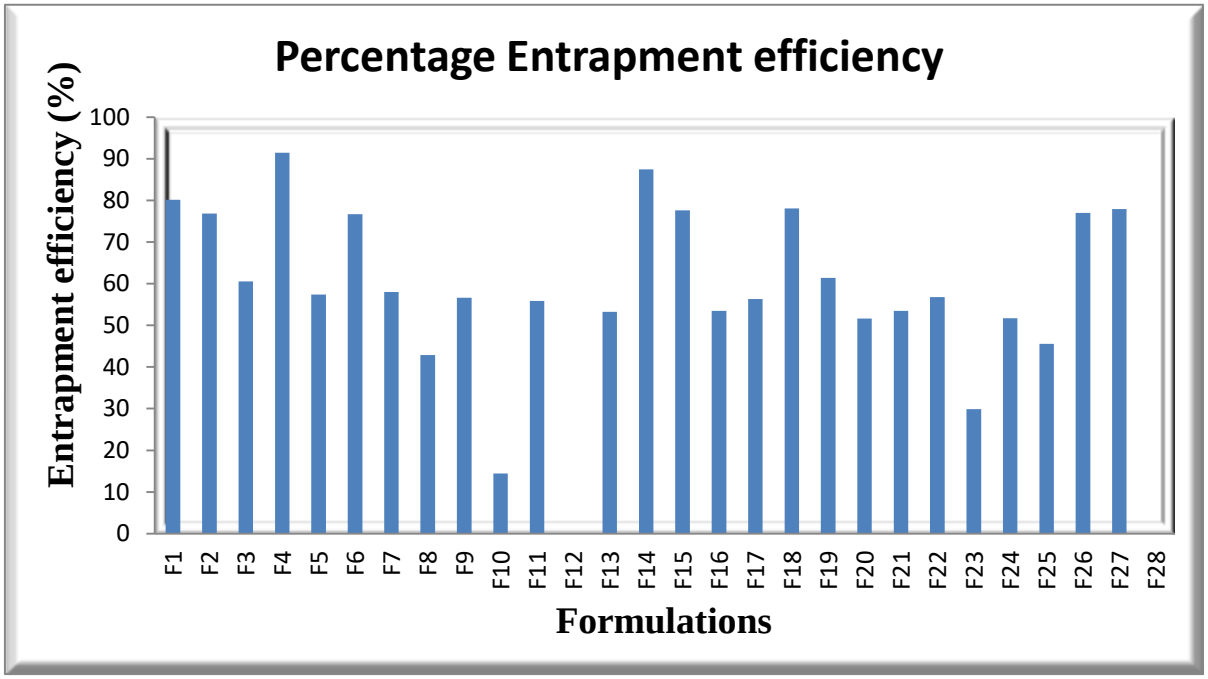


Fig 6.14. Percentage drug entrapment of different nano suspension formulations

6.6.5 In vitro drug permeation studies

In vitro drug permeation of formulation was performed for the prepared 28 formulations were shown in table 6.17. The drug permeation was absorbed for 90 minutes which also serves as one of the response in the optimization study. The results depicted the drug permeation for the formulation with the minimum release of 24.52 % for F_{23} and maximum permeation was 89.90 % for F_4 formulation. Although in formulation F_{12}, there was no permeation was observed for the drug due to absence of methanol in the formula. Additionally in case of F_{28} there was no permeation due to absence of drug in the formula. Therefore, the observed range of permeation include formulation F_{12} and F_{28} was within 24.52-89.90%.

Table 6.17

Drug permeated at 90 min for prepared formulations

Run. no.	Formulations	% Drug permeated at 90 min ± S.D
1	F_1	79.07 ± 2.248
2	F_2	69.80 ± 2.014
3	F_3	56.08 ± 1.458
4	F_4	89.90 ± 0.834
5	F_5	41.62 ± 1.043
6	F_6	62.09 ± 2..053
7	F_7	44.06 ± 2.022
8	F_8	31.45 ± 1.117
9	F_9	43.17 ± 2.858
10	F_{10}	58.80 ± 2.454
11	F_{11}	56.10 ± 2.357
12	F_{12}	00.00
13	F_{13}	66.63 ± 2.701
14	F_{14}	88.28 ± 2.237
15	F_{15}	75.74 ± 2.420
16	F_{16}	44.54 ± 2.461
17	F_{17}	68.75 ± 1.048
18	F_{18}	72.24 ± 1.873
19	F_{19}	41.18 ± 1.033
20	F_{20}	30.77 ± 1.415
21	F_{21}	32.50 ± 2.664
22	F_{22}	66.90 ± 1.866
23	F_{23}	24.52 ± 1.275
24	F_{24}	44.55 ± 2.466
25	F_{25}	33.08 ± 2.419
26	F_{26}	64.79 ± 2.838
27	F_{27}	80.58 ± 2.807
28	F_{28}	00.00

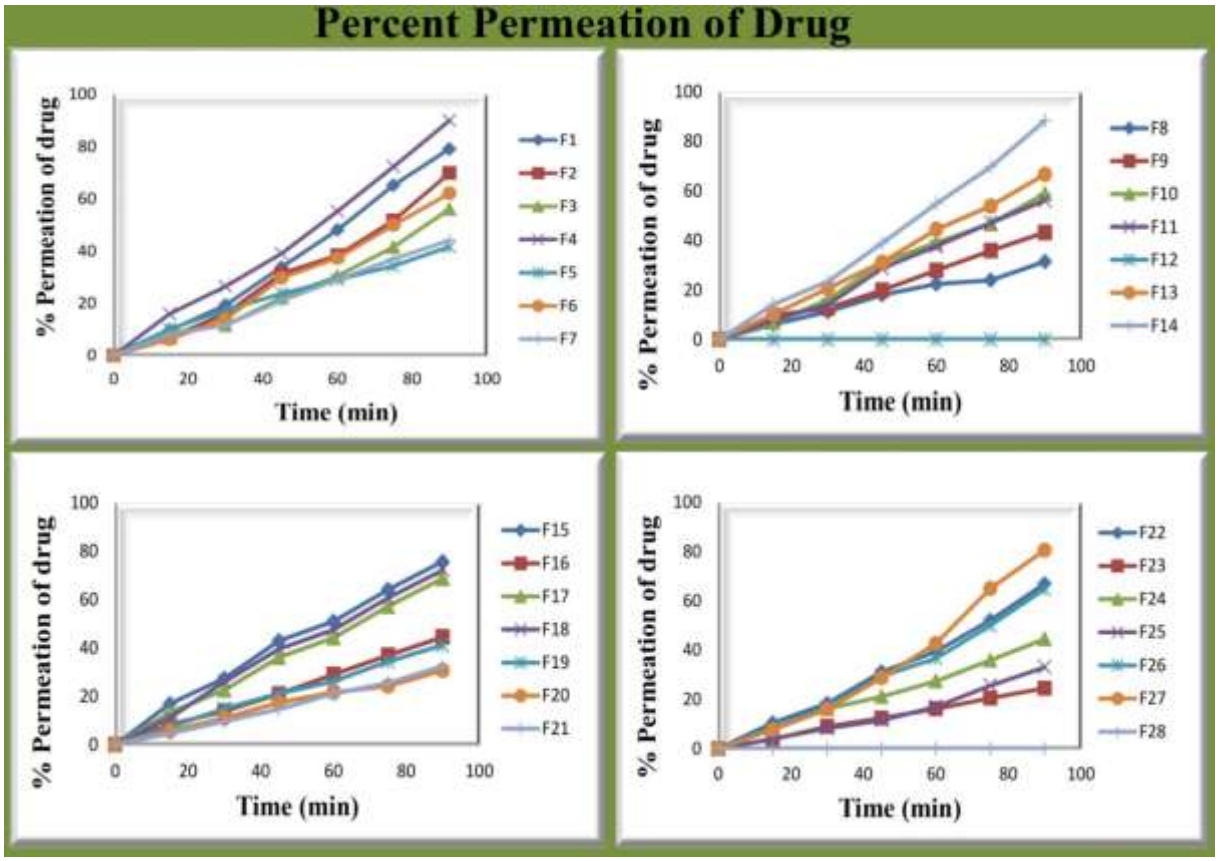


Fig. 6.15. Percent cumulative release of drug from different SLNs formulations

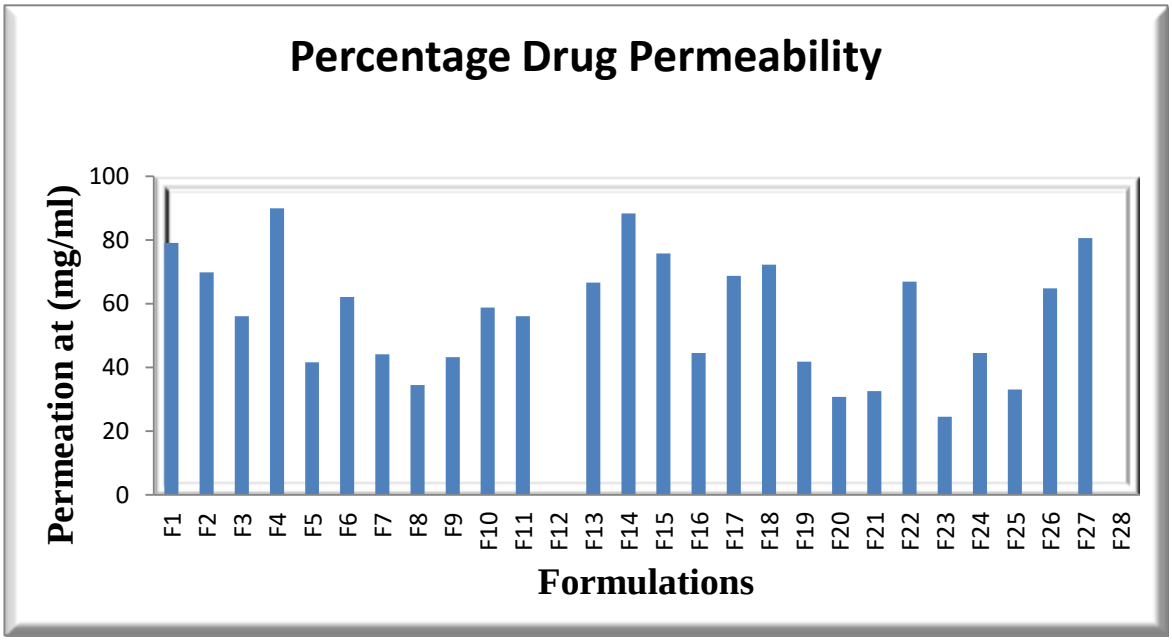


Fig. 6.16. Drug permeation study of nano suspension through membrane

6.7 Selection of optimized formulation

Based on the results of studies carried out to select suitable polymer, solvents and preparation method, different formulations were prepared. The formulations varied in terms of amount of drug, span 60, methanol, tween 80 and homogenization. A Central Composite Response Surface Rotatable Design was employed to obtain 28 different factor combinations and replicates where two independent variables were studied at two levels (Stat-Ease, 2011). Different factor combinations that were obtained and experimentally run to measure the responses Y_1 (percent entrapment efficiency) and Y_2 (percent permeability at 90 min) are given in table 6.18.

Figure 6.18 shows the FDS plot of the mean standard error over the design space. A fraction of design space (FDS) graph indicates the repeatability of experiment and possibility of detecting a significant effect. The FDS curve is the percentage of the design space volume containing a given standard error. The FDS graph in figure reveals a flatter and lower curve that means the overall prediction error will be constant and small. The value of FDS was found to be 0.938 which means that fraction of design space capable of predicting the true average within 1, standard deviation was 89.2%, which is higher than the recommended 80% value.

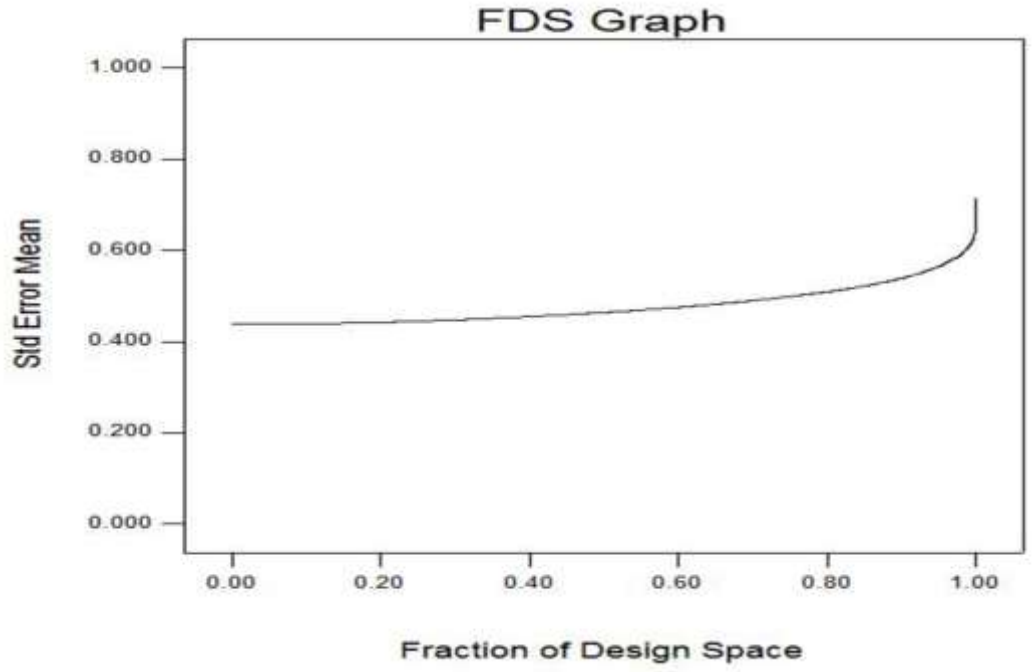


Fig. 6.17. Fraction of design space (FDS) graph for mean standard error

6.7.1 Statistical analysis

The formulations prepared according to the design were analyzed by using Design Expert®ver-10 software package. The effect of formulation variables on the response variables were statistically evaluated by one way ANOVA at 0.05 levels (Daniel 1983a; Stat-Ease 2011). The design was evaluated by response surface method using following polynomial equation 6.1:

$$Y=\beta_0 + \beta_1 A + \beta_2 B + \beta_3 AB \ldots\ldots\ldots \text{ (Equation 6.1)}$$

where, *Y* is the response variable, *β0* the constant and *β1, β2, β3* are the regression coefficients. *A* and *B* stand for the main effect, *AB* are the interaction terms and show how the response changes when two factors are simultaneously changed (Singh and Ahuja 2004; Singh, Kumar *et al.* 2004). The equation for each response parameter was generated using one way ANOVA and multiple linear response analysis (MLRA) (Daniel 1983b). A numerical optimization procedure using desirability approach was used to locate the optimal settings of the formulation variables in order to obtain the desired response. Constraints for the entrapment efficiency and percentage drug permeability were set in the range of 30 – 90.

In order to determine the significant design terms, their interactions and their effect on the response variables *Y1* and *Y2*, the design was evaluated by response surface analysis where the suitable model was selected on the basis of model *p*–values, lack of fit test, adjusted R2 and predicted R2. ANOVA was used to generate the quadratic (*Y1* and *Y3*) or linear (*Y2*) polynomial model equations. The model analysis values are given in table 6.18.

The models were found suitable for the response variables *Y1* and *Y2* were quadratic ($p<0.0001$) and quadratic ($p<0.0001$), regression models with R^2 values, respectively. All the lack of fit values was found to be insignificant ($p>0.05$) thus, indicating the validity of selected models. The closeness of adjusted R^2 (0.9893, 0.9849, 0.9786) and predicted R^2 (0.9521, 0.9623, 0.9351) to actual model R^2 (0. 0.9834, 0.9769 and 0.9738) also indicated the goodness of fit to the data. The observed values of R2 for selected models were close to 1.000 indicating excellent fit of the response surface polynomials to the response variable data. The adequate precision values ranged from 47.52 to 62.183, adequately higher than the required value of 4.000, indicating the precision of the results.

The optimization process predicted the optimized formulation by considering the ranges required for both the response factors. The best five batches priority wise shown by the

optimization were further prepared for carrying out validation. With the help of desirability plot of responses *i.e.* for entrapment efficiency and percentage drug permeation for variable factors drug, span 60, methanol, tween 80 and homogenization, optimized formulation was selected. The criteria in order of priority were highest entrapment efficiency and high percentage permeability (Raza et al., 2010; Sheo et al., 2010; Song et al., 2012). The fitting of terms in the polynomial equation indicated that the model was significant and would navigate effectively through the design space. Final polynomial equations for each response variable in terms of coded factors are given below:

Y_1 = 88.71 + 8.81 * A - 7.93 * B + 8.11 * C + 11.28 * D - 3.14 * E - 2.24 * AB + 2.91* AC + 1.42 * AD - 3.25 * AE - 3.56 * BC + 2.16 * BD - 5.16 * BE + 3.74 * CD - 3.80 * CE - 1.65 * DE - 3.32 * A^2 + 5.29 * B^2 + 2.54 * C^2 + 8.31 *D^2 + 9.20 * E^2…….…… (Equation 6.2)

Y_2 = 92.71+5.26* A-4.34* B+6.42* C+5.61* D-1.47* E-7.41* AB+1.58* AC+4.14* AD+3.04* AE-3.01* BC-6.94* BD+3.58* BE+6.13* CD-2.04* CE-5.44* DE+1.33* A^2+1.11* B^2-8.69* C^2+4.84* D^2+14.89* E^2……..……(Equation 6.3)

Final polynomial equations for each response variable in terms of actual factors are given below:

Y_1 = 18.51+214.16* Drug-194.52* Span 60-34.35* Methanol-5.61* Tween 80-21.526* Homogenization-894.25* Drug * Span 60+582.15* Drug * Methanol+56.72* Drug * Tween 80-6.49* Drug * Homogenization-9.37* Span 60 * Methanol-864.75* Span 60 * Tween 80-1011.25* Span 60 * Homogenization+74.87* Methanol * Tween 80-3799.38* Methanol * Homogenization-330.37* Tween 80 * Homogenization-1328.94* $Drug^2$+211.55* $Span\ 60^2$-7.25* $Methanol^2$+33.24* $Tween\ 80^2$+919.89* $Homogenization^2$……(Equation 6.4)

Y_2 = 63.3-20.59* Drug+146.37* Span 60+1128.83* Methanol-299.05* Tween 80-12.85* Tween 80-128.59* Homogenization-2.96* Drug * Span 60+3.17* Drug * Methanol+16.56* Drug * Tween 80+60.80* Drug * Homogenization-602.50* Span 60 * Methanol-27.75* Span 60 * Tween 80+7.15* Span 60 * Homogenization+1225.23* Methanol * Tween 80-204.11* Methanol * Homogenization-10.87* Tween 80 * Homogenization+533.25* $Drug^2$+44.42* $Span\ 60^2$-869.37* $Methanol^2$+1936.7* $Tween\ 80^2$+48.86* $Homogenization^2$...(Equation 6.5)

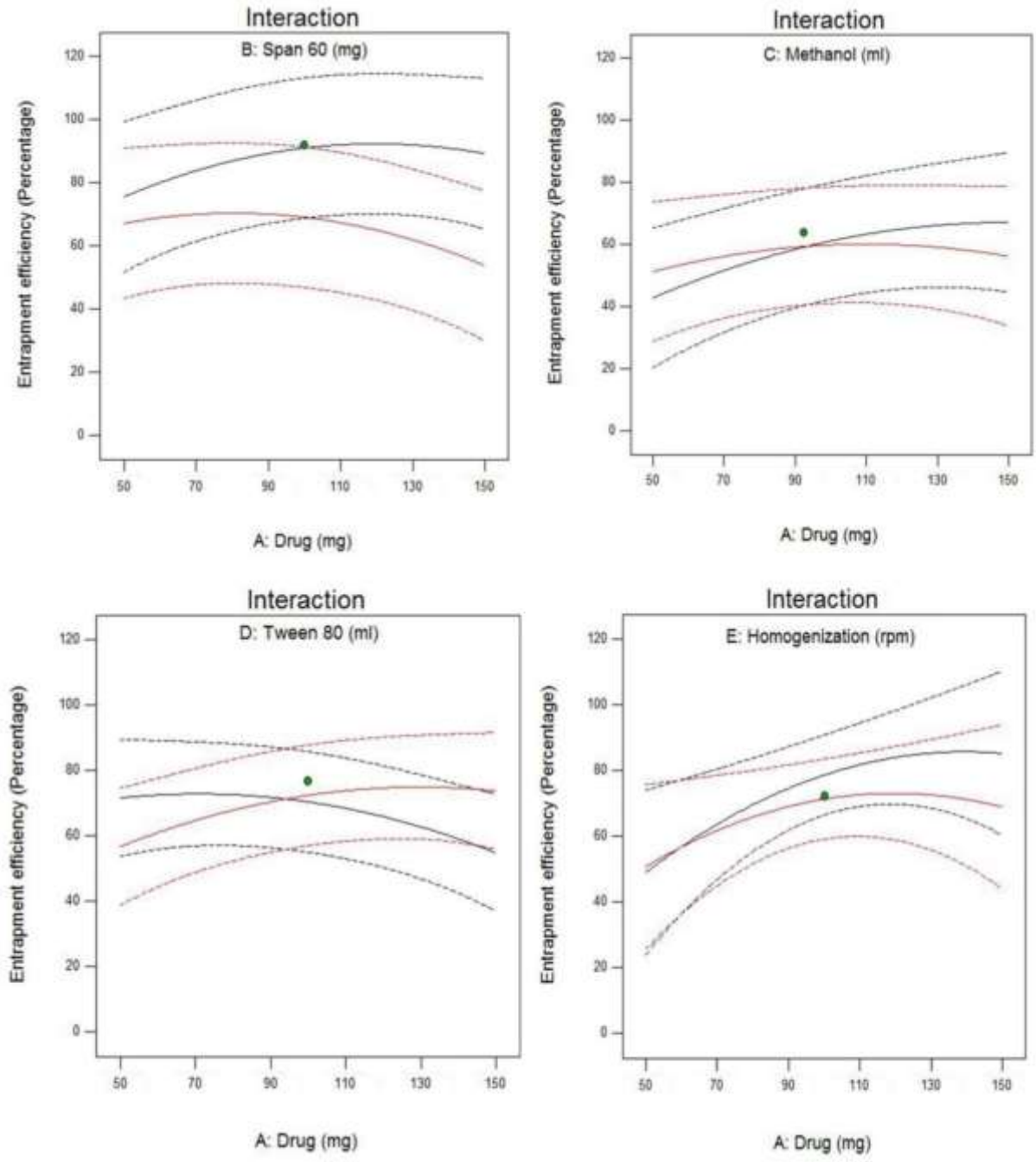
Response surface graphs, interaction plots, contour plots for entrapment efficiency and percentage drug permeation as per CCD were obtained from Design Expert® software.

Fig. 6.19, 6.20 6.21 and 6.22 showed interaction plots and contour plots for entrapment efficiency respectively. In the same way, Fig. 6.23, 6.24 6.25 and 6.26 represented interaction plots and contour plots for percentage drug permeability.

Table 6.18

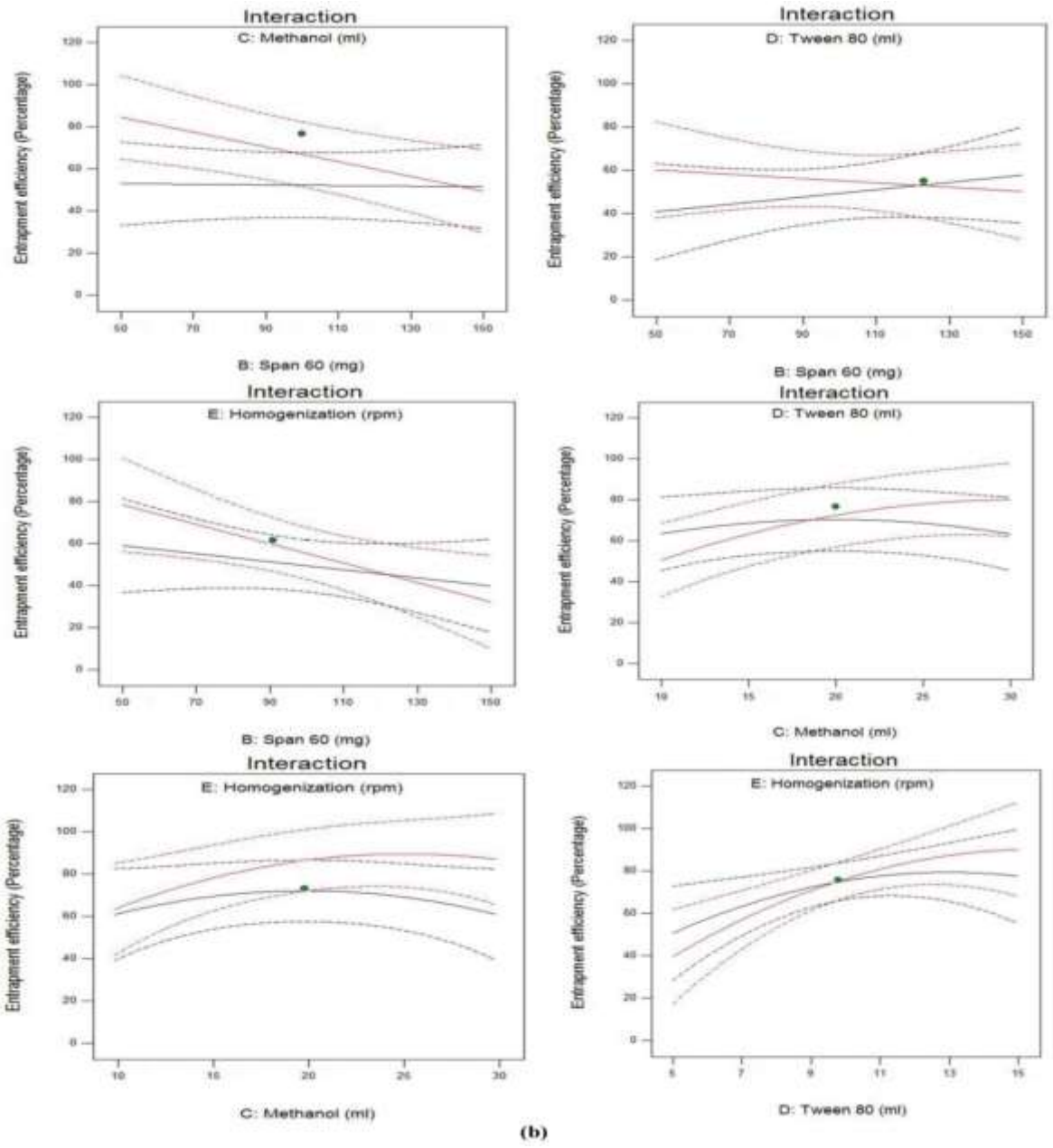
Statistical parameters for different response variables obtained by ANOVA and multi linear regression analysis

Coefficients (Factor)	Entrapment efficiency (Y1)	Permeability of drug (Y2)
(β_0) Intercept	88.71*(p< 0.0001)*	92.71*(p< 0.0001)*
(β_1) A-Drug (mg)	8.813*(p< 0.0001)*	5.258*(p< 0.0007)*
(β_2) B-Span 60 (mg)	-7.930*(p< 0.0001)*	-4.342*(p< 0.0012)*
(β_3) C-Methanol (ml)	8.110*(p< 0.0001)*	6.418*(p< 0.0027)*
(β_4) D-Tween 80 (ml)	11.279*(p< 0.0001)*	5.575*(p< 0.0094)*
(β_5) E-Homogenization (rpm)	-3.135*(p< 0.0064)*	-1.472*(p< 0.0004)*
(β_6) AB	-2.236*(p< 0.0787)*	-7.414*(p< 0.0065)*
(β_7) AC	2.913*(p< 0.0072)*	1.584*(p< 0.0003)*
(β_8) AD	1.418*(p< 0.0065)*	4.141*(p< 0.0093)*
(β_9) AE	-3.246*(p< 0.0002)*	3.040*(p< 0.0001)*
(β_{10}) BC	-3.469*(p< 0.0006)*	-3.013*(p< 0.0006)*
(β_{11}) BD	-2.162*(p< 0.0032)*	6.938*(p< 0.0044)*
(β_{12}) BE	-5.056*(p< 0.0187)*	3.575*(p< 0.0035)*
(β_{13}) CD	3.744*(p< 0.0051)*	6.125*(p< 0.0509)*
(β_{14}) CE	-3.799*(p< 0.0012)*	-2.040*(p< 0.0004)*
(β_{15}) DE	-1.652*(p< 0.0008)*	-5.438*(p< 0.0031)*
(β_{16}) A^2	-3.322*(p< 0.0495)*	1.333*(p< 0.0028)*
(β_{17}) B^2	5.289*(p< 0.0056)*	1.111*(p< 0.0001)*
(β_{18}) C^2	2.543*(p< 0.0005)*	-8.694*(p< 0.0009)*
(β_{19}) D^2	8.310*(p< 0.0088)*	4.842*(p< 0.0578)*
(β_{20}) E^2	9.199*(p< 0.0022)*	4.887*(p< 0.0035)*
R-Squared	0.983	0.976
Adj R-Squared	0.989	0.984
Pred R-Squared	0.952	0.962
Adeq Precision	42.47	55.71
Lack of Fit	F= 4.10665653*(p< 0.1351)*	F= 3.78320114*(p< 0.3711)*
Model		
Linear	***	***
Quadratic	F=94.56 (p<0.0010)	F=90.10 (p<0.0021)



(a)

Fig. 6.18. (a) Interaction plots for the terms A and B with respect to response variable Y_1 while the term C,D,E is constant at all levels of A and B
Interaction plots for the terms A and C with respect to response variable Y_1 while the term B,D,E is constant at all levels of A and C
Interaction plots for the terms A and D with respect to response variable Y_1 while the term B, C, E is constant at all levels of A and D
Interaction plots for the terms A and E with respect to response variable Y_1 while the term B, C, D is constant at all levels of A and E



(b)

Fig. 6.19. (b) Interaction plots for the terms B and C with respect to response variable Y_1 while the term A,D,E is constant at all levels of B and C
Interaction plots for the terms B and D with respect to response variable Y_1 while the term A,C,E is constant at all levels of B and D
Interaction plots for the terms B and E with respect to response variable Y_1 while the term A, C, D is constant at all levels of B and E
Interaction plots for the terms C and D with respect to response variable Y_1 while the term A, B, E is constant at all levels of C and D
Interaction plots for the terms C and E with respect to response variable Y_1 while the term A,B,D is constant at all levels of C and E
Interaction plots for the terms D and E with respect to response variable Y_1 while the term A, B,C is constant at all levels of D and E

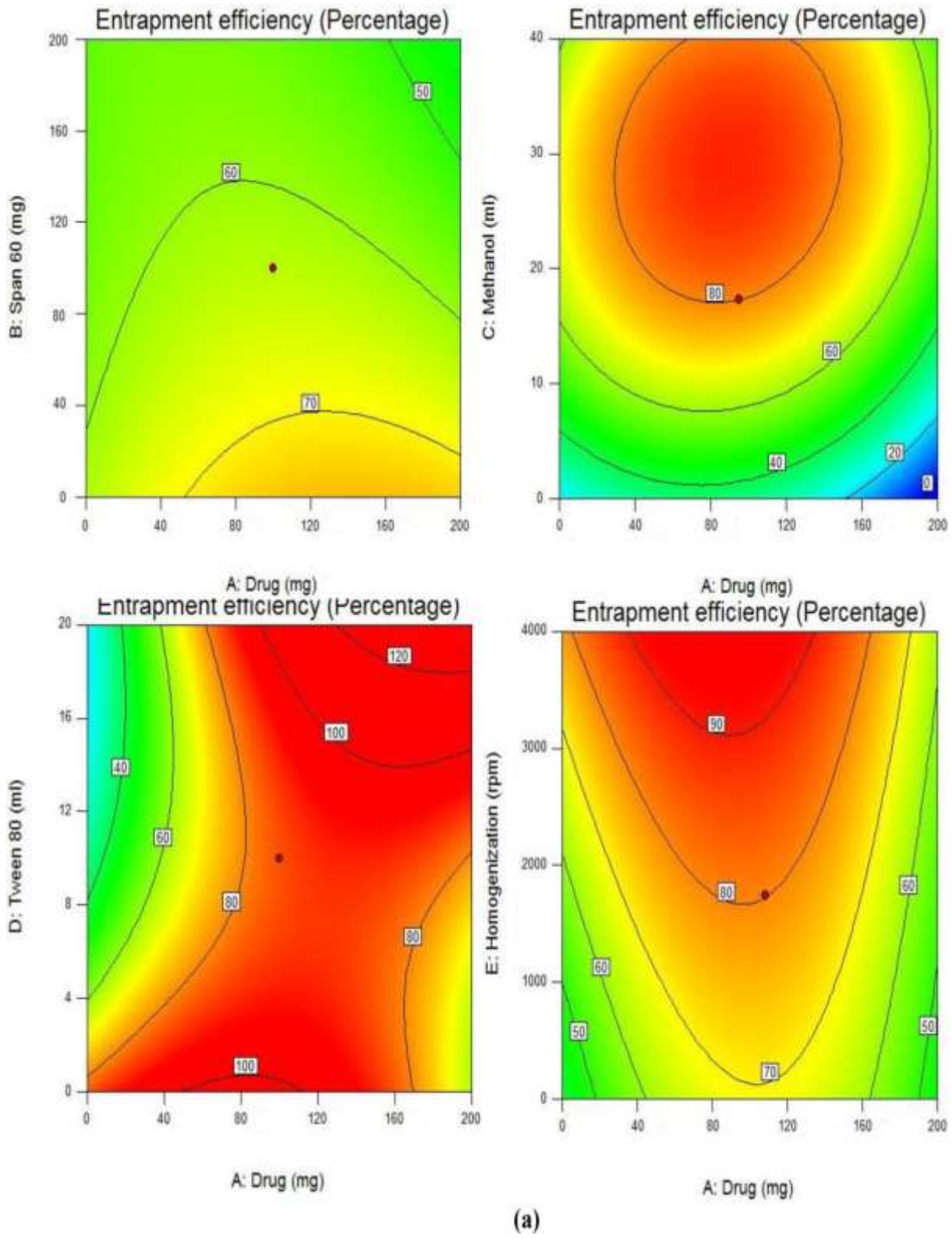
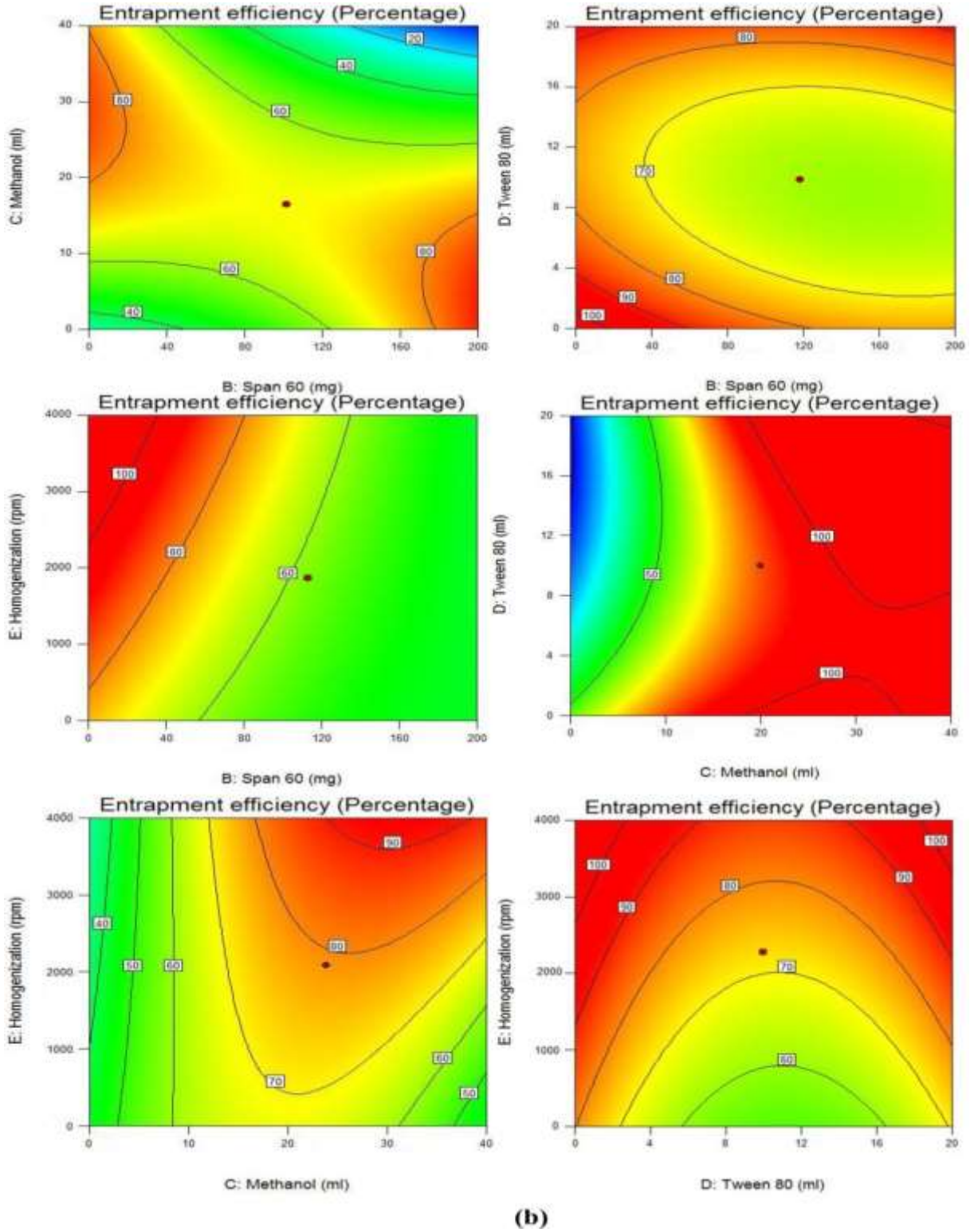


Fig.6.20. (a) Representative contour plots of Entrapment efficiency as per CCD Drug: Span 60 (AB), Drug: Methanol (AC), Drug: Tween 80 (AD), Drug: Homogenization (AE)

RESULTS AND DISCUSSION



(b)

Fig.6.21. (b) Representative contour plots of Entrapment efficiency as per CCD Span 60: Methanol (BC), Span 60: Tween 80 (BD), Span 60: Homogenization (BE), Methanol: Tween 80 (CD), Methanol: Homogenization (CE), Tween 80: Homogenization (DE),

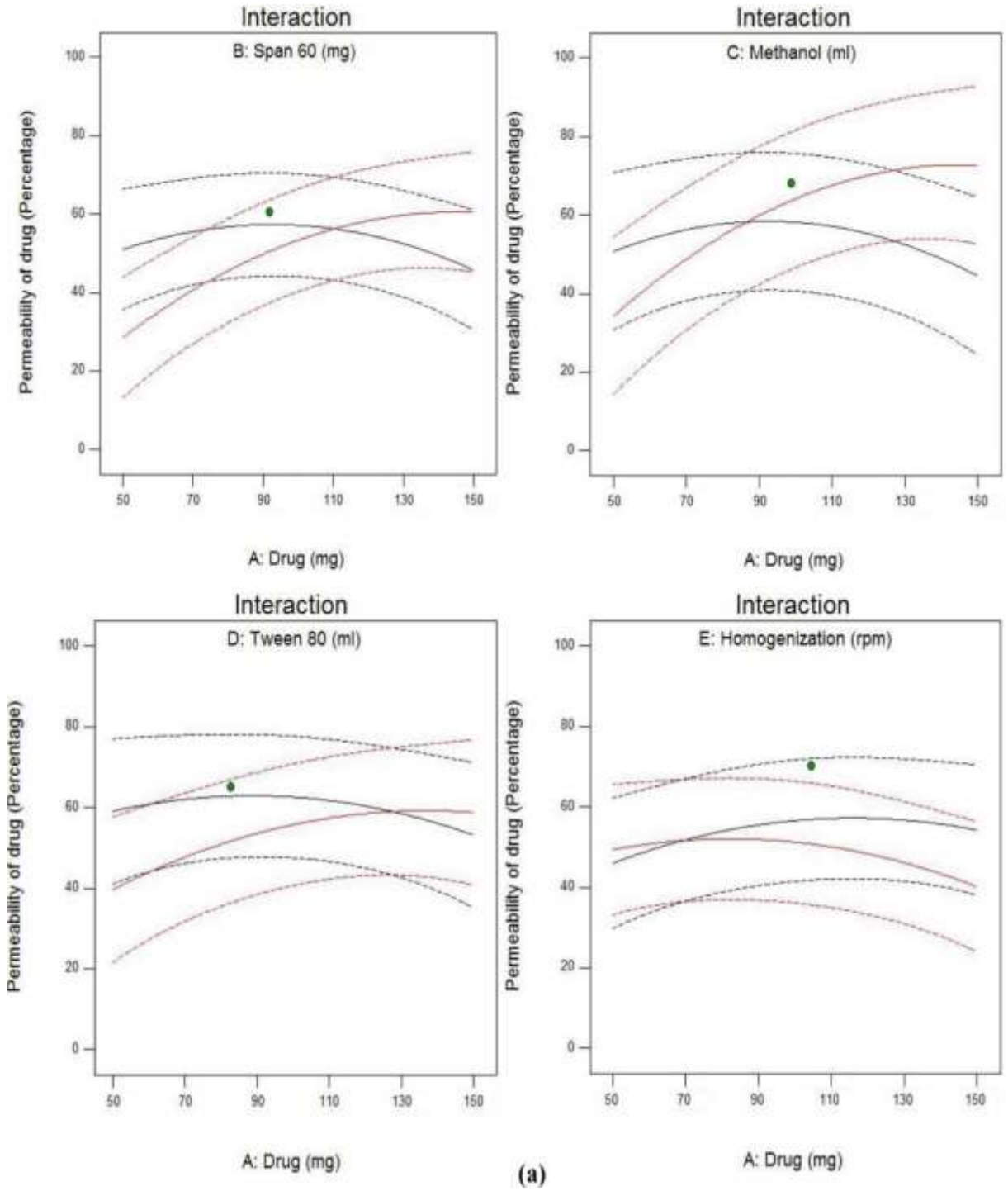


Fig. 6.22. (a) Interaction plots for the terms A and B with respect to response variable Y_2 while the term C,D,E is constant at all levels of A and B
Interaction plots for the terms A and C with respect to response variable Y_2 while the term B,D,E is constant at all levels of A and C
Interaction plots for the terms A and D with respect to response variable Y_2 while the term B, C, E is constant at all levels of A and D
Interaction plots for the terms A and E with respect to response variable Y_2 while the term B, C, D is constant at all levels of A and E

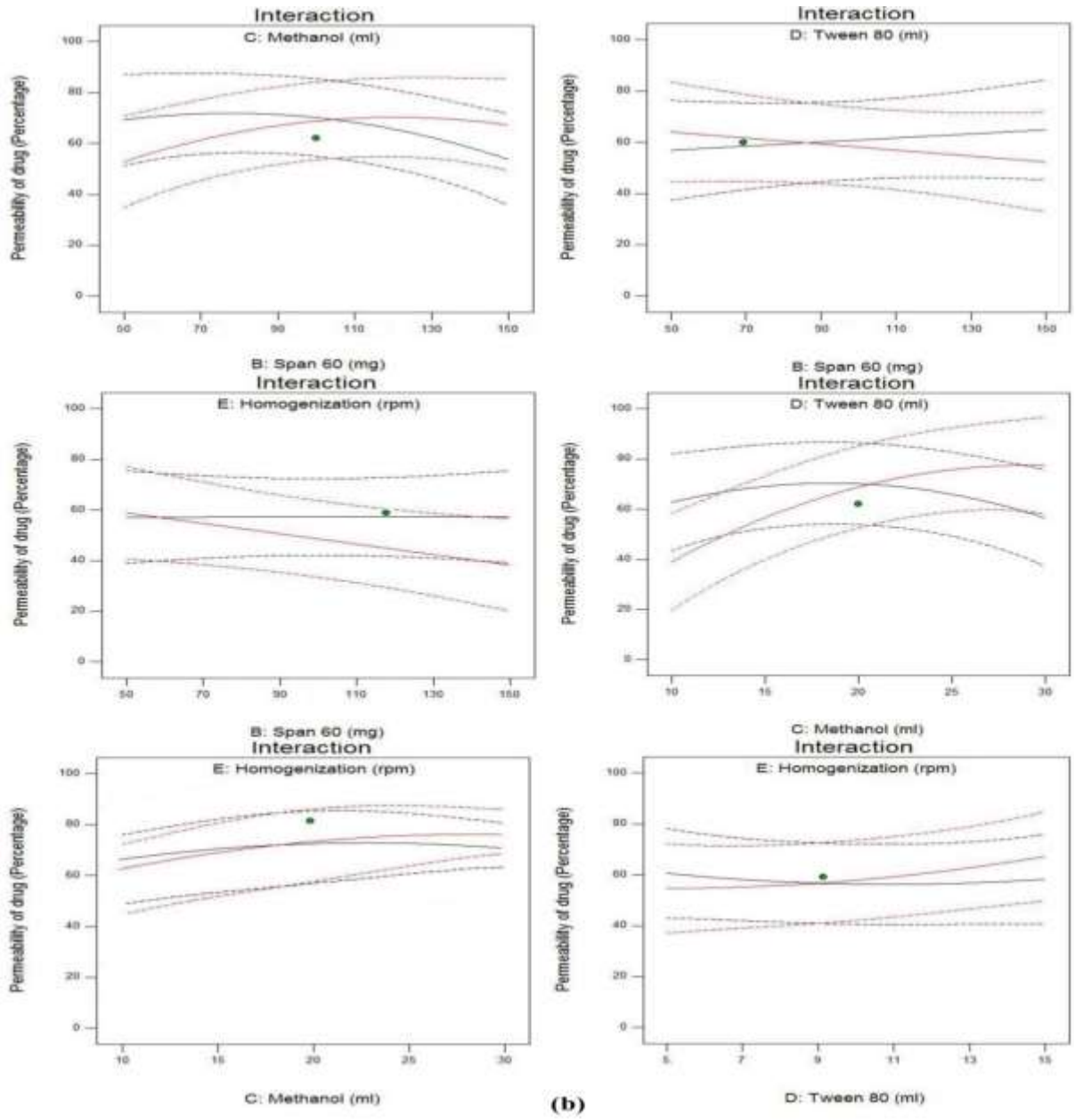


Fig. 6.23. (b) Interaction plots for the terms B and C with respect to response variable Y_2 while the term A,D,E is constant at all levels of B and C
Interaction plots for the terms B and D with respect to response variable Y_2 while the term A,C,E is constant at all levels of B and D
Interaction plots for the terms B and E with respect to response variable Y_2 while the term A, C, D is constant at all levels of B and E
Interaction plots for the terms C and D with respect to response variable Y_2 while the term A, B, E is constant at all levels of C and D
Interaction plots for the terms C and E with respect to response variable Y_2 while the term A,B,D is constant at all levels of C and E
Interaction plots for the terms D and E with respect to response variable Y_2 while the term A, B,C is constant at all levels of D and E

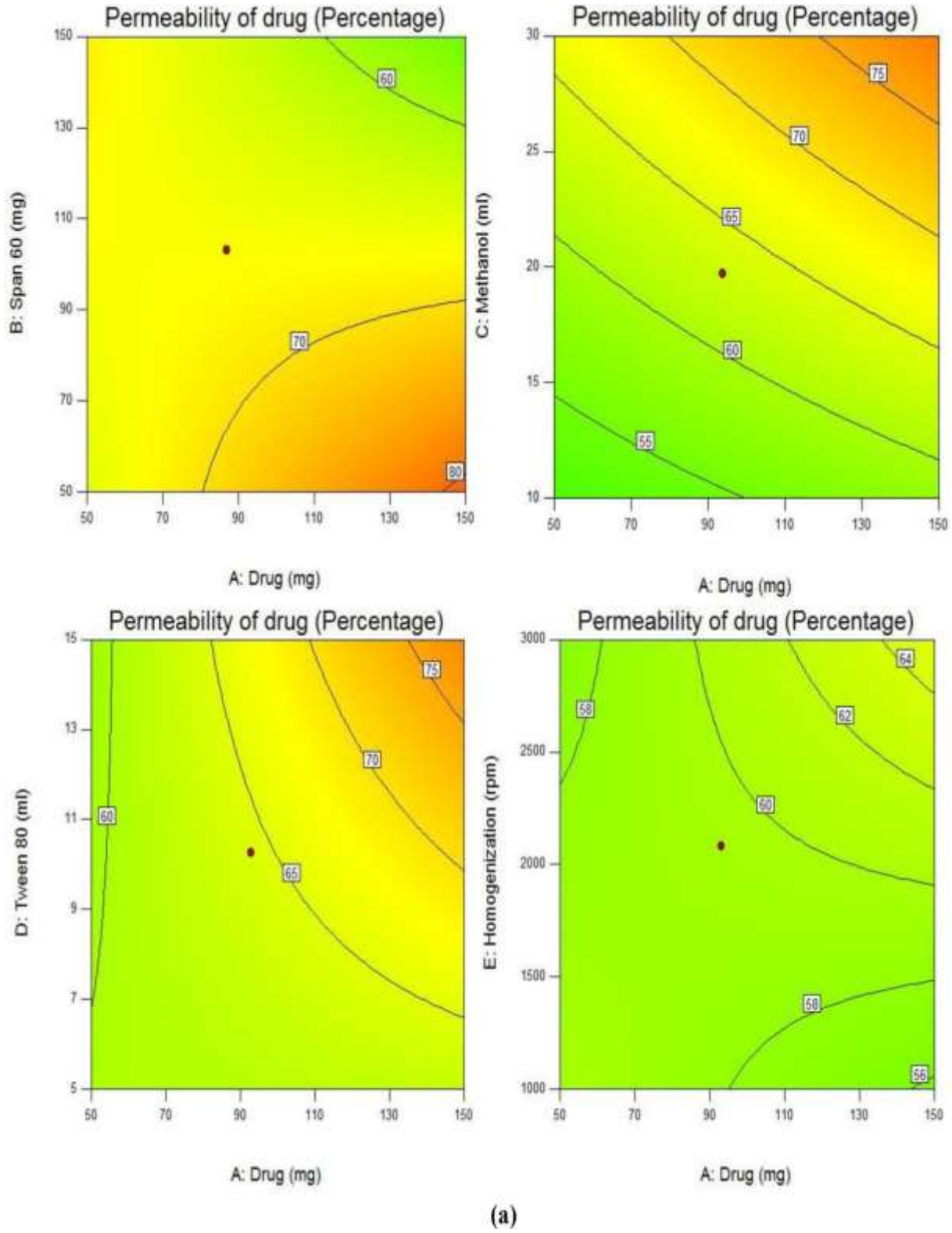


Fig. 6.24. (a) Representative contour plots of Permeability of Drug as per CCD Drug: Span 60 (AB), Drug: Methanol (AC), Drug: Tween 80 (AD), Drug: Homogenization (AE)

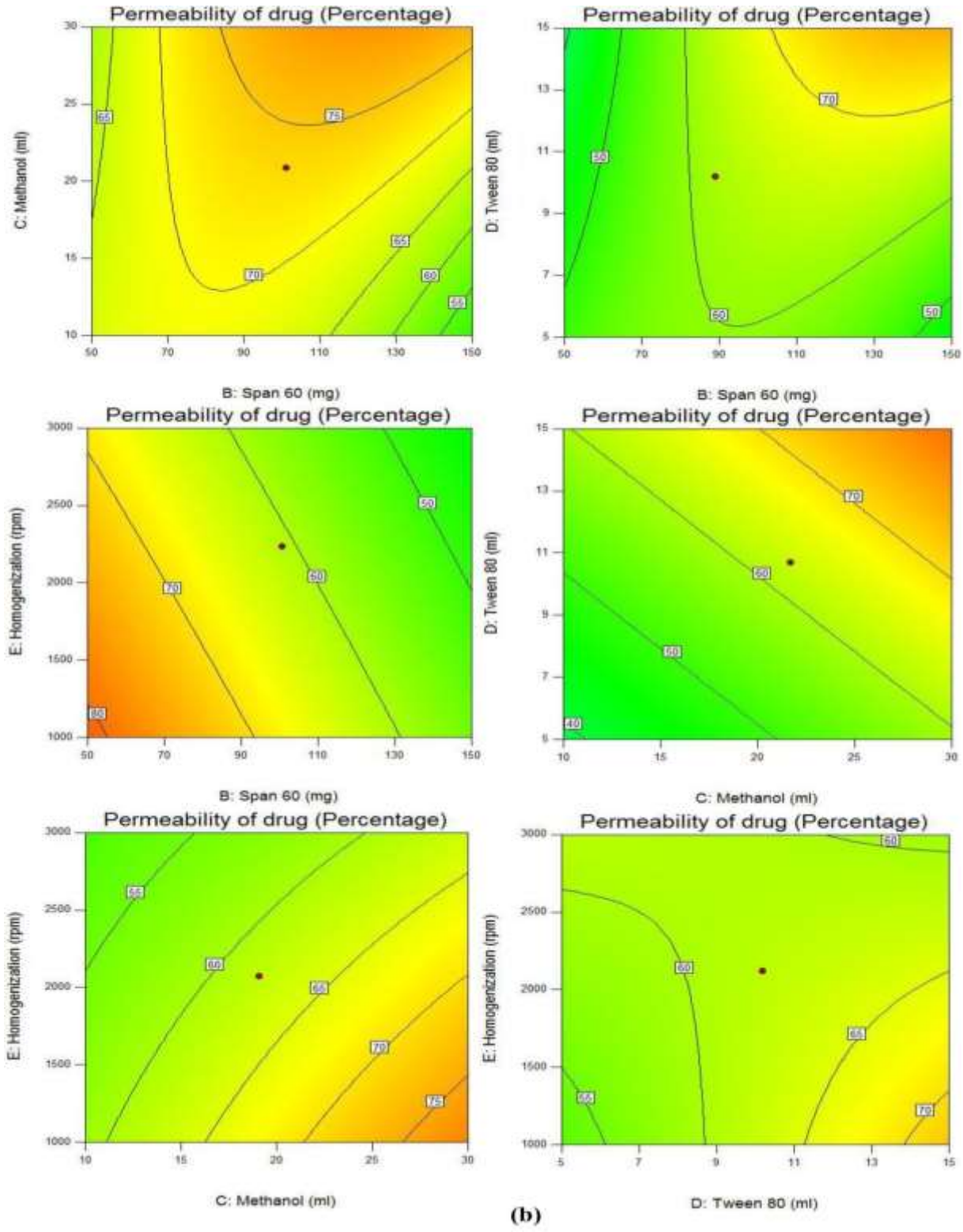


Fig. 6.25. (b) Representative contour plots of Permeability of Drug as per CCD Span 60: Methanol (BC), Span 60: Tween 80 (BD), Span 60: Homogenization (BE), Methanol: Tween 80 (CD), Methanol: Homogenization (CE), Tween 80: Homogenization (DE),

6.7.2 Validation of Optimized results

The optimization design suggested 47 best optimized batches after processing, out of which best five were considered as shown in table 6.18. Comparison of experimental and predicted responses, *i.e.* entrapment efficiency and drug permeation as per DoE is listed in table 6.19. The percentage error ranged between -1.75 and 3.02 (table 6.19). These data showed that most of the predicted values are close to the experimental values. These indicated the prognostic ability of the nano suspension formulation of Itraconazole using systematic optimization via CCD was validated. The optimized formulation obtained by numeric optimization was validated for its performance by preparing all the four resulting formulations thrice.

Table 6.19

Validation of optimized batch of SLNs dispersion

Formulation Code	Drug (mg)	Span 60 (mg)	Methanol (ml)	Tween 80 (ml)	Homogenization (rpm)
V1	150.00	50.00	30.00	15.00	1000.00
V2	120.26	50.00	29.42	15.00	1000.00
V3	132.11	50.01	30.00	14.55	1032.43
V4	150.00	62.91	30.00	14.75	1000.00
V5	150.00	50.07	27.14	14.72	1000.00

Table 6.20

Comparison of Experimental results with predicted values with percentage error

Formulation Code	Response	Predicted value	Experimental value	Percentage error
V1	Entrapment efficiency (%)	92.34	91.48	0.86
	Permeability of drug (%)	90.54	89.9	0.64
V2	Entrapment efficiency (%)	83.69	85.44	-1.75
	Permeability of drug (%)	77.31	74.29	3.02
V3	Entrapment efficiency (%)	82.20	83.21	-1.01
	Permeability of drug (%)	77.71	79.22	-1.51
V4	Entrapment efficiency (%)	80.56	78.92	1.64
	Permeability of drug (%)	78.62	75.88	2.74
V5	Entrapment efficiency (%)	77.94	76.32	1.62
	Permeability of drug (%)	79.83	77.31	2.52

The optimized and validated nano suspension dispersion V1 with the composition as shown in table 6.18 was analyzed on the basis of statistical parameters. 3-D plots for entrapment efficiency and permeability of drug as shown in Fig. 6.27 6.28 6.29 and 6.30 respectively, represents the optimized range of the components, Itraconazole, span 60, methanol, tween 80 and homogenization, which can provide the best possible entrapment efficiency and permeability of drug in combination. The plots help to define the relationship between the components by observing the response surface. The optimized nano suspension dispersion offered percentage entrapment efficiency of 79.8 % and percentage drug permeability of 90.36 %. The observed responses were the best optimal responses in combination as compared to other suggested responses.

The 3-D plots along clearly defined the optimal range of the nano suspension formulation. The optimized formulation was found to offer the best optimal responses in the form of percentage entrapment efficiency and percentage drug permeability. The repeatability and robustness of the preparation of nano suspension dispersion and evaluation of responses were carried out. It was ensured that on the basis of Design Expert® software and statistical parameters, the optimized formulation was the best formulation as compared to other formulations which were suggested by the software.

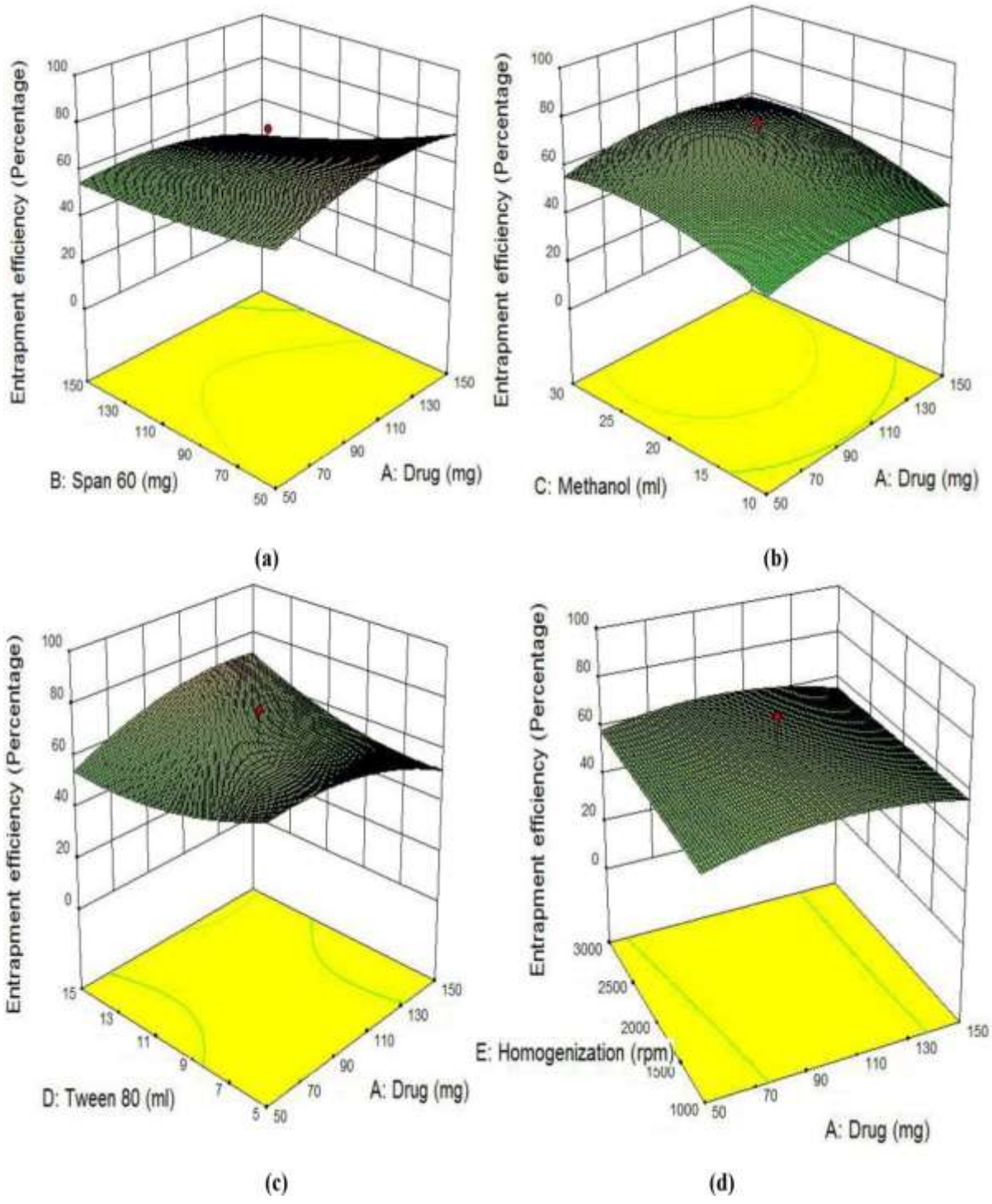


Fig. 6.26. 3-D plots of optimized nano suspension formulation (V1) for Entrapment efficiency (a) Plot between drug and span 60 keeping methanol, tween 80, and homogenization constant, (b) Plot between Drug and methanol keeping span 60, tween 80, and homogenization constant, (c) Plot between Drug and tween 80 keeping span 60, methanol, and homogenization constant, (d) Plot between Drug and homogenization keeping span 60, methanol, and tween 80 constant.

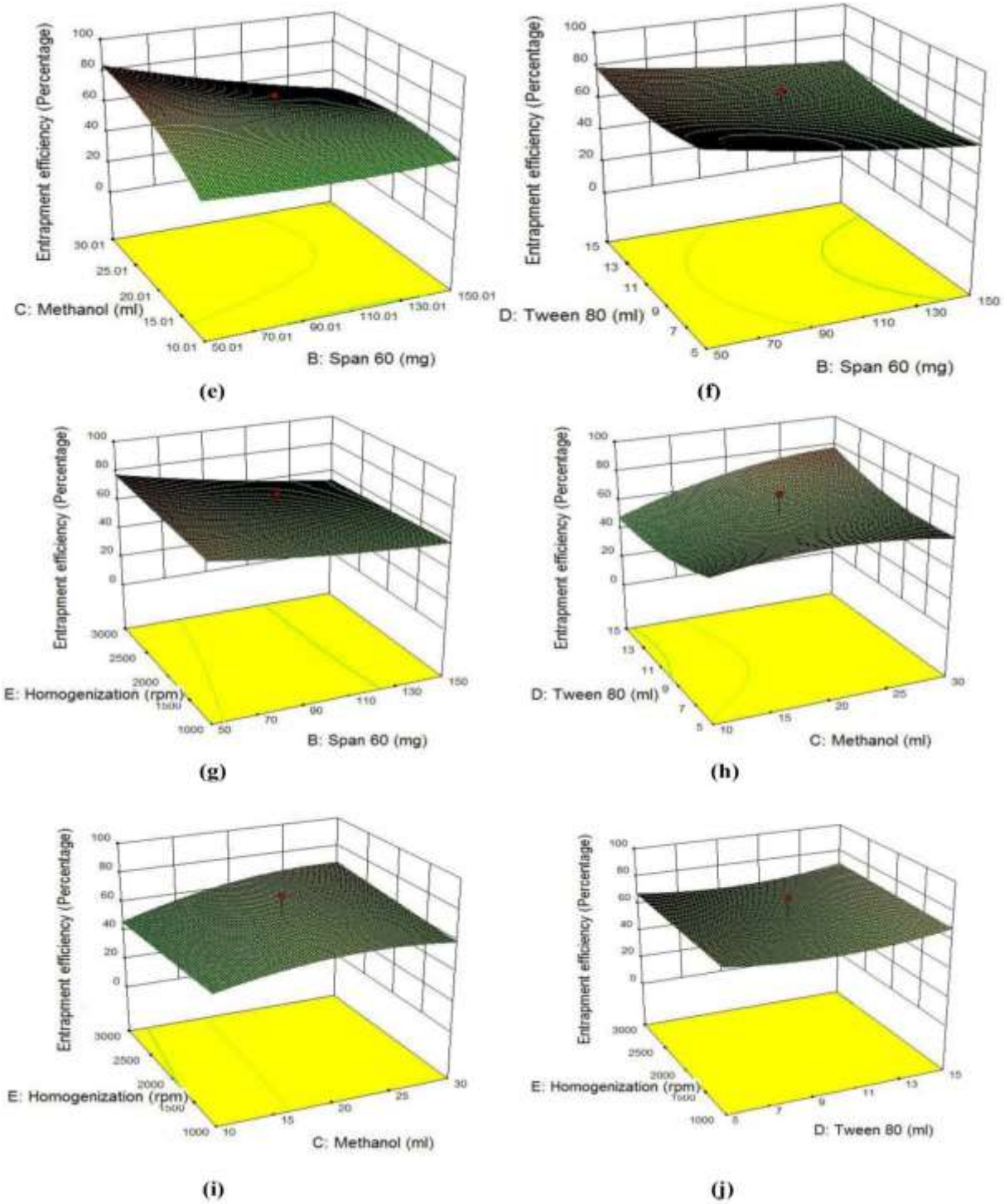


Fig. 6.27. 3-D plots of optimized nano suspension formulation (V1) for Entrapment efficiency (e) Plot between span 60 and methanol keeping drug, tween 80, and homogenization constant, (f) Plot between span 60 and tween 80 keeping drug, methanol, and homogenization constant, (g) Plot between span 60 and homogenization keeping drug, methanol, and tween 80 constant, (h) Plot between methanol and tween 80 keeping drug, span 60, and homogenization constant, (i) Plot between methanol and homogenization keeping drug, span 60, and tween 80 constant, (j) Plot between tween 80 and homogenization keeping drug, span 60, and methanol constant.

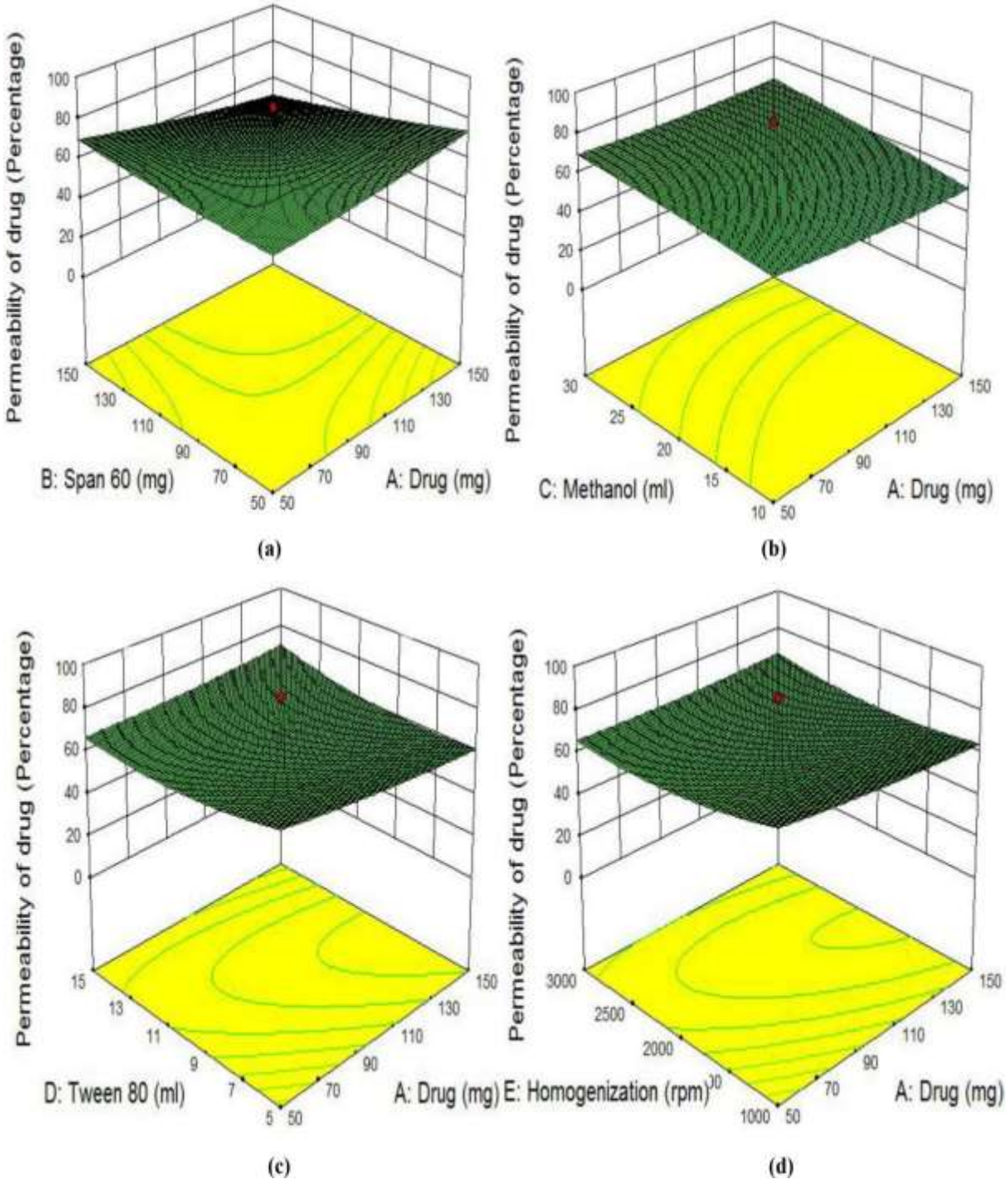


Fig. 6.28. 3-D plots of optimized nano suspension formulation (V1) for permeability of drug (a) Plot between drug and span 60 keeping methanol, tween 80, and homogenization constant, (b) Plot between Drug and methanol keeping span 60, tween 80, and homogenization constant, (c) Plot between Drug and tween 80 keeping span 60, methanol, and homogenization constant, (d) Plot between Drug and homogenization keeping span 60, methanol, and tween 80 constant.

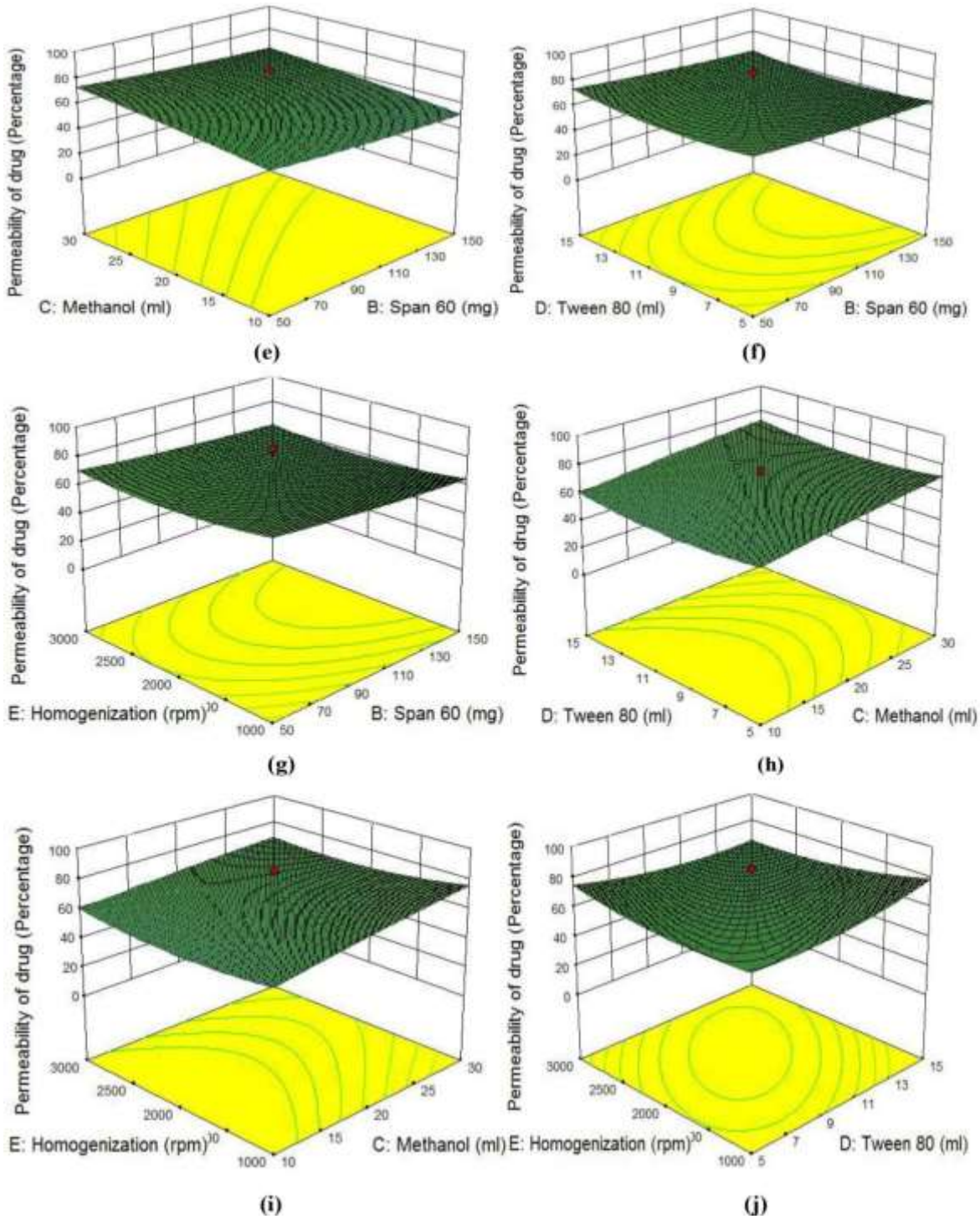


Fig. 6.29. 3-D plots of optimized nano suspension formulation (V1) for Entrapment efficiency (e) Plot between span 60 and methanol keeping drug, tween 80, and homogenization constant, (f) Plot between span 60 and tween 80 keeping drug, methanol, and homogenization constant, (g) Plot between span 60 and homogenization keeping drug, methanol, and tween 80 constant, (h) Plot between methanol and tween 80 keeping drug, span 60, and homogenization constant, (i) Plot between methanol and homogenization keeping drug, span 60, and tween 80 constant, (j) Plot between tween 80 and homogenization keeping drug, span 60, and methanol constant

6.7.2.1 Morphological study of optimized SLNs formulation

Transmission electron microscopy (TEM) image of optimized nano suspension formulation is shown in Fig. 6.28. The results obtained from drug loaded optimized nano suspension formulation showed the morphology of spherical particles. The smallest particle size observed was 10-150 nm at magnification of 19000 X. It could be observed from the TEM image that the formed particles were spherical in nature, thus, confirming the nano suspension formulation.

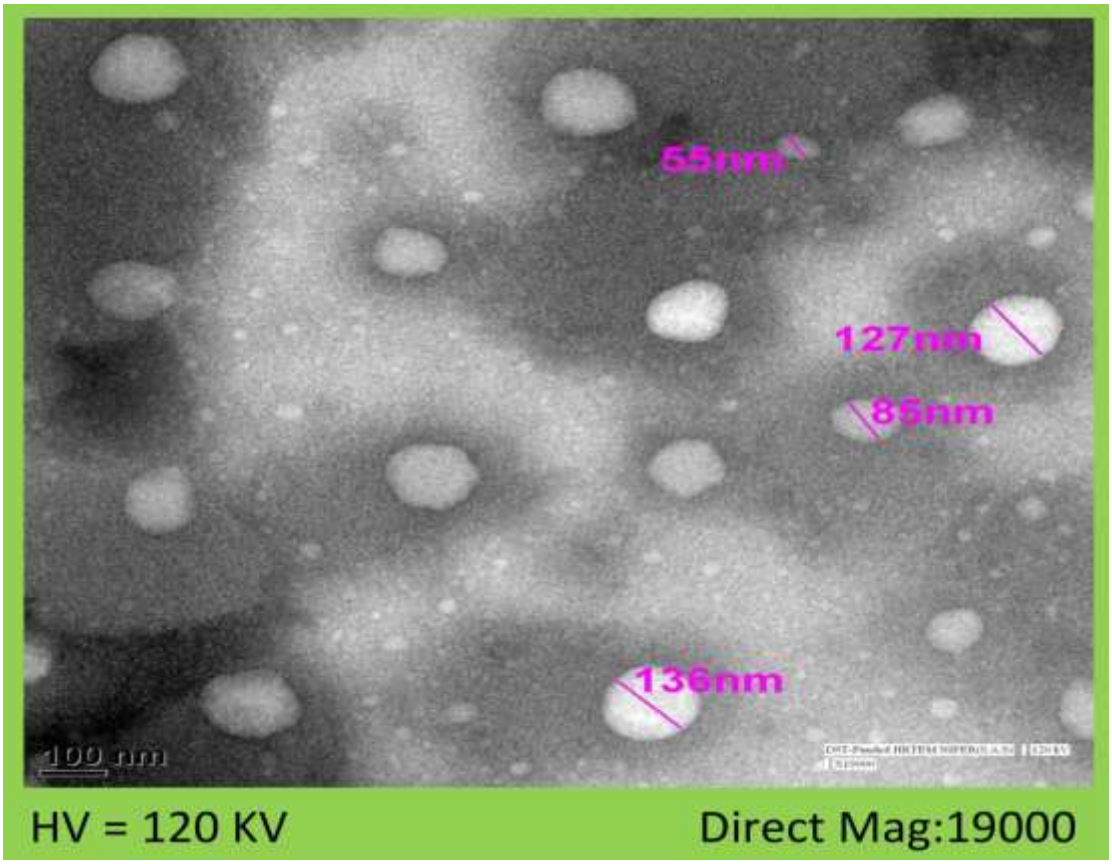


Fig. 6.30. TEM image of optimized nano suspension formulation (V1) at 19000 X

6.7.2.2 Stability study of optimized nano suspension *particle dispersion*

Table 6.20 and Fig.6.29 represents the stability data of optimized nano suspension dispersion at 4 ± 3°C and 50 ± 2°C for 15 days. The data showed that there was negligible loss (0.17 %) of entrapped drug at 5 ± 3°C after 15 days of storage. Whereas at 25 ±2°C, comparatively more loss of drug (1.76%) occurred but it was within the limits so the system can be considered as stable. Thus the results showed that the particles are stable at room temperature. As it was observed that the nano suspension were more stable at refrigerated

conditions, so it appears that it is better to provide the same conditions to provide more stability.

Table 6.21

Percentage encapsulated drug loss from optimized cubosomes at different temperature depicting stability study

Time (in days)	Entrapment efficiency (%) at 5 ± 3°C	Entrapment efficiency (%) at 25 ± 2°C	Encapsulated drug loss (%) at 5 ± 3°C	Encapsulated drug loss (%) at 25 ± 2°C
0	91.48	91.48	0	0
2	91.48	91.22	0	0.26
6	91.35	90.88	0.13	0.60
10	91.32	90.26	0.16	1.22
15	91.31	89.72	0.17	1.76

6.8 Evalution of Itraconazole loaded shampoo:

6.8.1 Physical appearance or visual inspection:

The appearance of shampoo is one of the important aspects to be considered. The formulated shampoo preparations (S_1-S_6) were evaluated for physical characteristics such as color, odor, transparency and nature. There were variation in the features but were not significant as shown in table 6.22.

Table: 6.22

Physical evalution of formulated shampoo preparations

Formulations	Color	Transparency	Odor	Nature
S_1	Light white	Transparent	Good	Gelly but not sticky
S_2	white	Milky	Pungent	Gelly but more sticky
S_3	Light white	Transparent	Good	Viscous
S_4	white	Milky	Pungent	More gelly but not sticky
S_5	white	Opaque	Pungent	Slightly Gelly but sticky
S_6	white	Opaque	Pungent	Liquid
Marketed shampoo	pink	Opaque	Pungent	Liquid

6.8.2 pH:

The pH of the shampoo is usually kept within slightly alkaline to neutral range to avoid irritation to scalp and eyes. The pH of prepared Itraconazole shampoo formulations was observed by using the pH meter (Systronic, μ pH system, India). The pH formulations was from 4.53-5.21. Therefore, by referring to literature and available reports, the shampoo with pH range for the (S_6) 5.21 can be considered good which was shown in table 6.23. The pH profile for the formulations were compared with the marketed shampoo which was found to be 5.42

Table: 6.23

pH profile of shampoo formulations

Formulations	pH
S_1	4.95
S_2	4.72
S_3	4.78
S_4	4.53
S_5	5.07
S_6	5.21
Marketed shampoo	5.42

6.8.3 Rheology

Rheological investigations are basically concerned with determination of the relationship between shear stress, stress rate and viscosity (Plaizier-Vercammen, Lecluse *et al.* 1989). Power law equation is widely applicable. It is given as:

$\gamma = Ktn$……. (Equation 6.6)

Taking logarithm on both the sides

$Log\ \gamma = Log\ K + n\ Log\ t$ …….... (Equation 6.7)

Where, τ is shear stress, γ is shear rate, *K* is consistency index and *n* is flow index.

Consistency index and flow index are calculated by plotting graphs of log shear rate *v.s* log shear stress (Kapil, Kapoor *et al.* 2009). Flow index (dimensionless) is a measure of the deviation from Newtonian behavior (n =1), $n < 1$ indicates shear thinning (pseudoplastic behavior) and $n > 1$ shear thickening (dilatant behavior). The viscosity of the optimized

formulation and marketed formulation was determined at 37°C with cup and bob rheometer using approximate 15 of sample as shown in Fig 6.32 and 6.33. Viscosity was determined at various shear rates by putting the shampoo to various torque values as shown in table 6.24. Measurement of each sample was done over a range of 2- 100 s-1 of the shear rate. The plot of shear stress Vs shear strain was obtained. Similarly a plot of shear rate Vs viscosity was plotted. The equation was applied to power law. It was observed that both the shampoo followed pseudoplastic behaviour along with the viscosities which were equivalent for both the marketed as well as optimized Itraconazole shampoo.

Table 6.24

Rheological parameters of marketed and optimized Itraconazole shampoo

Formulation	n	Viscosity (Pa.S)	Consistency
Marketed shampoo	0.784	3.77- 49.1	109.56
Optimized shampoo	0.736	3.54 – 48.7	105.6

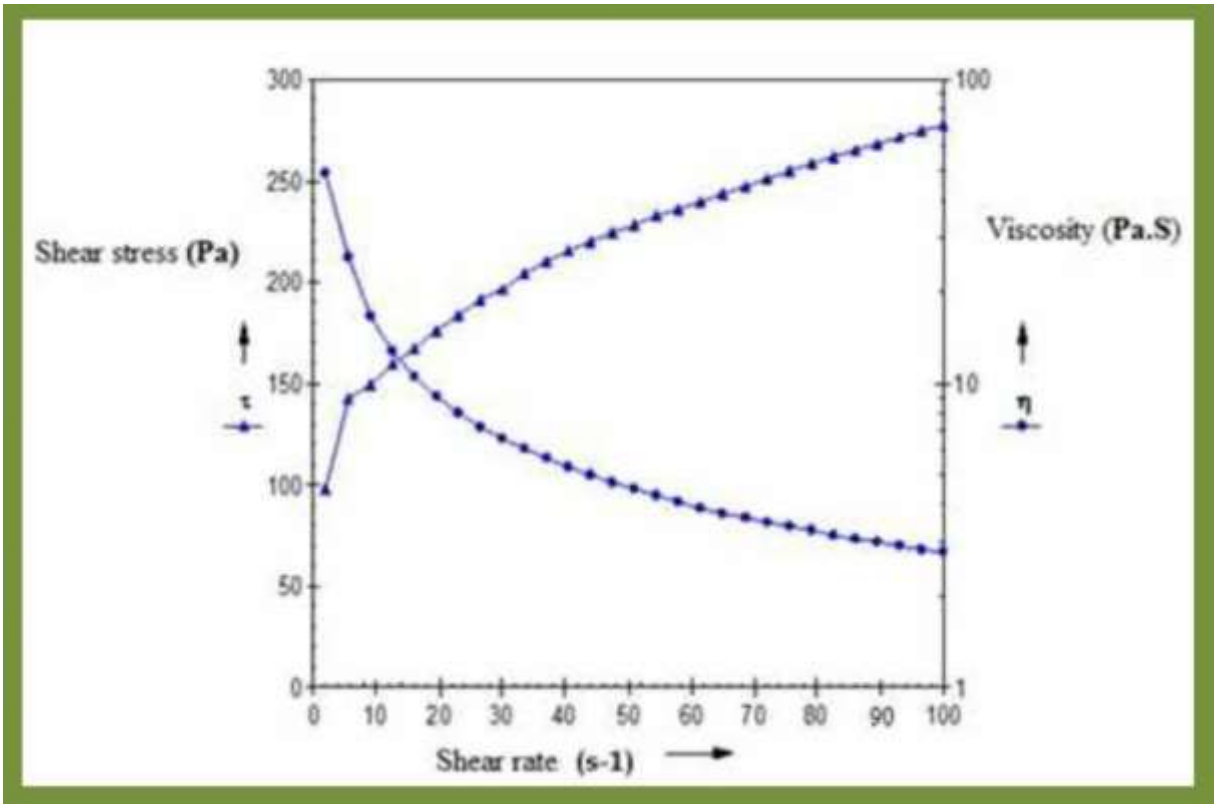


Fig. 6.31. Plot showing the viscosity and shear stress at various shear rate of marketed shampoo formulation

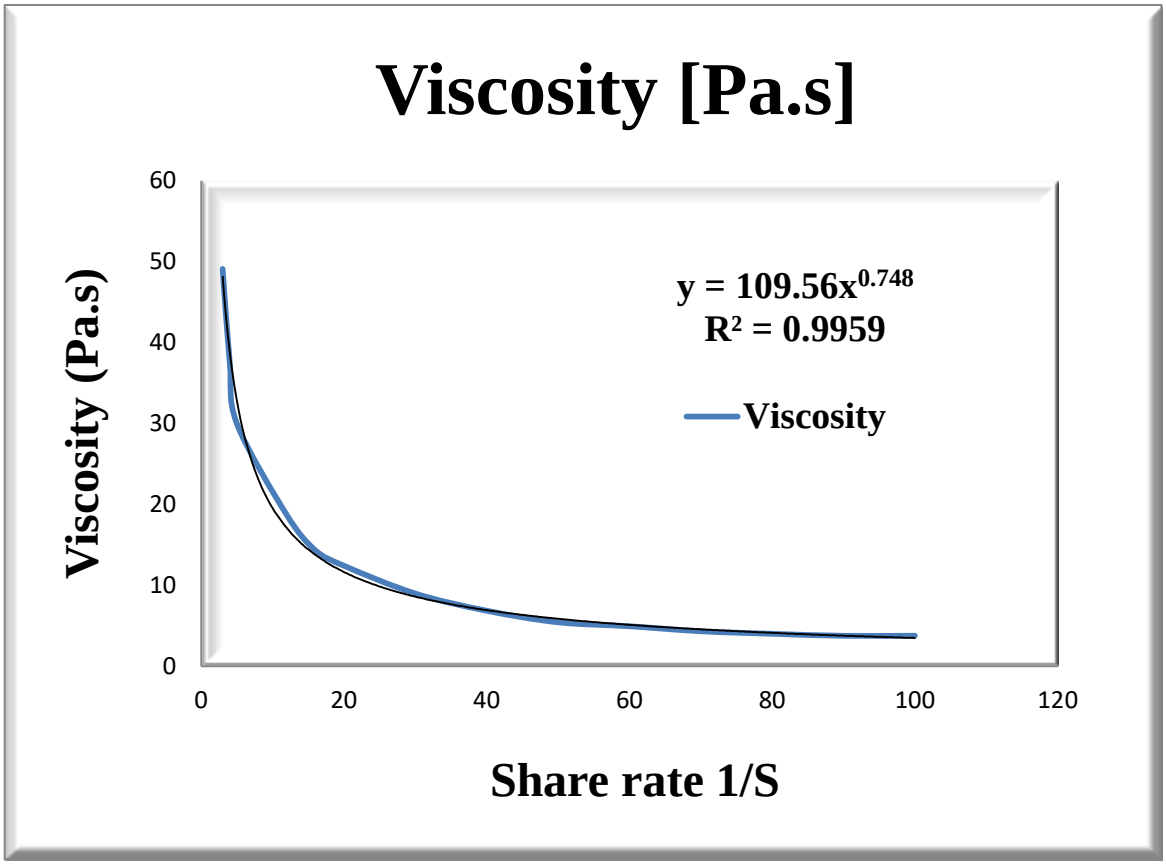


Fig. 6.32. Plot showing the viscosity of marketed shampoo at different shear rate

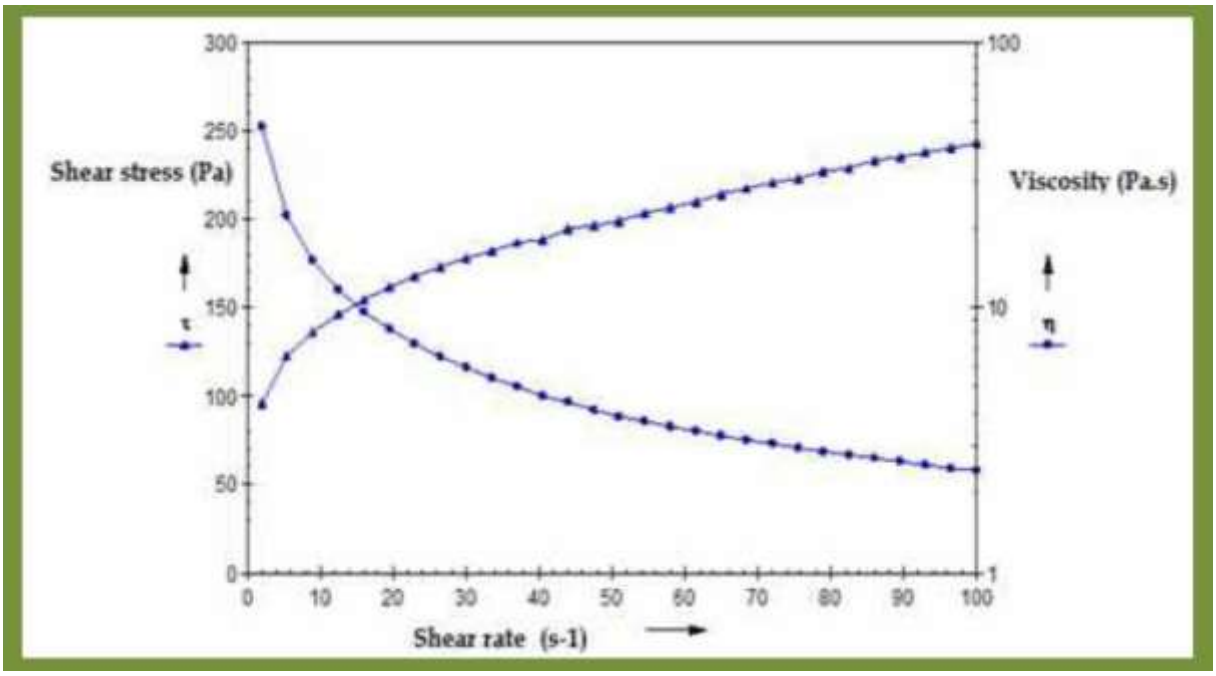


Fig. 6.33. Plot showing the viscosity and shear stress at various shear rate of Optimized shampoo formulation

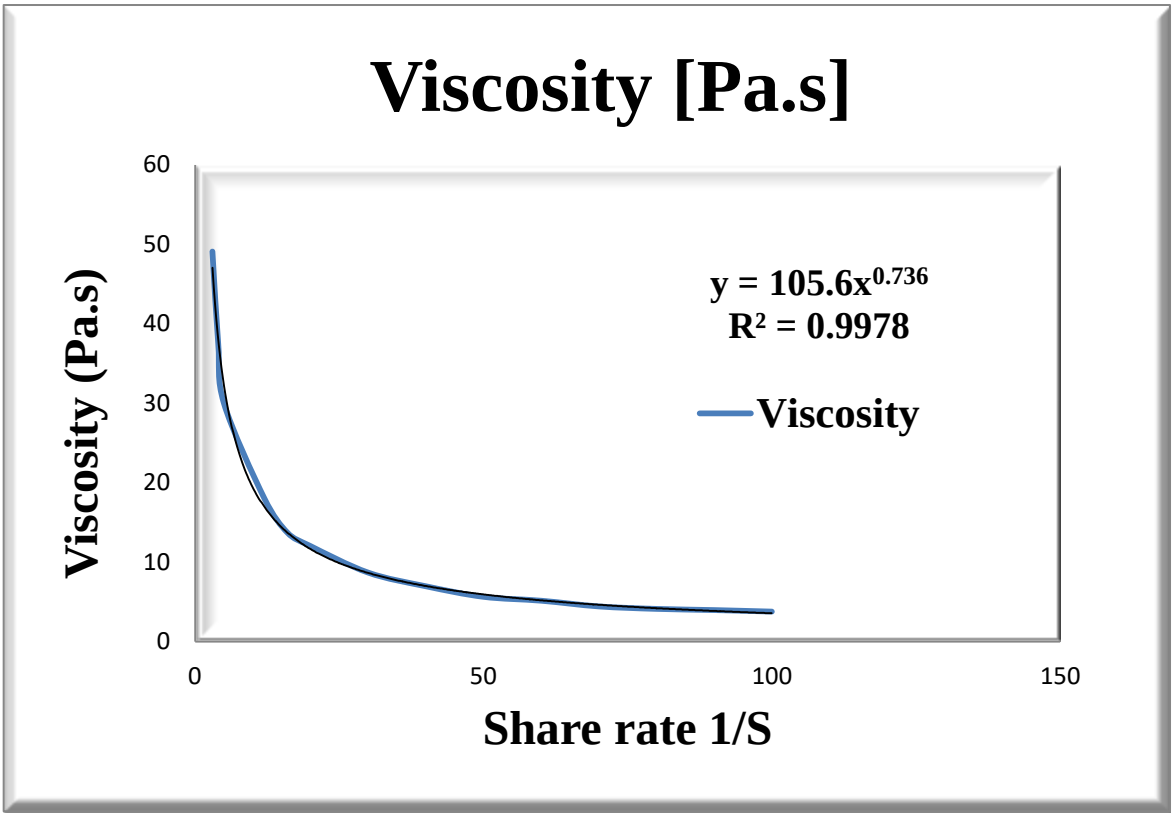


Fig. 6.34. Plot showing the viscosity of formulated shampoo at different shear rate

6.8.4 Foaming ability and Foam stability:

The foaming index was determined to evaluate the ability of prepared shampoo to provide foam when applied. The foaming ability in shampoo is associated with the detergency ability for better cleaning. Therefore, the foaming index was calculated for the six formulations of shampoo, out of which, formulation S_6 offered best related to foaming. The results were then compared with the foaming ability of marketed shampoo which was shown in table 6.25.

Table: 6.25

Foaming ability of shampoo formulations

Formulations	Foaming index (ml)
S_1	152
S_2	149
S_3	154
S_4	150
S_5	156
S_6	161
Marketed shampoo	160

6.8.5 Drug content and content uniformity

For analysis of uniform drug distribution, the samples of Itraconazole shampoo were taken from 3 to 4 separate points and were determined spectrophotometrically. Table 6.26 depicts the results for content uniformity as the drug content was found to be within the range of 97-99 % so the prepared shampoo formulation containing Itraconazole can be considered as homogeneously dispersed.

Table 6.26

Drug content of Itraconazole shampoo

S. no.	Expected amount of drug in shampoo (mg)	Quantified amount of drug (mg)	Drug content (%)
1	150	145.5	97
2	150	148.5	99
3	150	147.0	98

6.8.6 In vitro drug permeation studies:

In vitro drug permeation study was prepared for the prepared shampoo preparations to analyse the actual amount of drug reaching to the site of action. Although the shampoo is meant for the superficial application but the permeation ensures retention and prolonged effect of drug. Therefore, the cumulation permeation of drug was recorded and compared with the permeation profile of marketed antifungal shampoo.

Table 6.27.1

Percentage permeation of Itraconazole from optimized formulations and marketed ketoconazole shampoo

Time (min)	% Drug permeated at 90 min mg/ ml ± S.D			
	S_1	S_2	S_3	S_4
0	0	0	0	0
15	19.56±1.230	19.06±0.434	21.17±2.477	21.78±1.219
30	31.89±2.201	30.65±2.530	32.75±1.538	34.11±2.157
45	46.56±0.613	44.18±1.836	43.29±1.844	48.78±0.589
60	55.67±1.666	58.66±1.304	60.77±1.343	57.89±1.634
75	69.01±2.113	70.25±2.385	72.35±1.408	71.22±2.073
90	76.78±1.373	80.67±1.821	82.99±2.052	79.01±2.626

Table 6.27.2

Percentage permeation of Itraconazole from optimized formulations and marketed ketoconazole shampoo

Time (min)	% Drug permeated at 90 min mg/ ml ± S.D		
	S_5	S_6	M.S
0	0	0	0
15	22.22±2.011	28.86±1.429	32.89±1.516
30	33.91±1.993	42.02±1.207	45.22±1.090
45	46.87±2.153	53.82±0.916	59.89±1.875
60	64.35±1.765	70.88±0.633	69.00±1.773
75	74.88±1.245	84.04±1.064	82.33±1.129
90	85.41±1.935	96.47±1.202	93.22±1.144

6.9 Selection of best shampoo formulations

Depending on the evalution parameters, the best shampoo formulation was identified. Out of the six formulations, the results obtained for the S_6 were better, therefore, it was considered to be best shampoo formulations, comparable to the marketed shampoo. Furthermore, stability, a drug release kinetic model and in-vitro antifungal study were conducted for the finalized shampoo formulations and was compared with the marketed shampoo.

6.10 Stability study of formulated shampoo

The stability study of formulated shampoo was carried at different temperatures i.e. 4-8°C (Refrigerator; RF) and 50 ± 2°C (Room temperature; RT) for a period of 15 days as shown in table 6.27. The change in pH along with the drug content of shampoo was checked at various time interval and the results revealed that the shampoo was stable over both the conditions with small changes. The prepared shampoo thus, can be considered stable.

Table 6.28

Stability study of SLNs shampoo at different temperature conditions

Time (in days)	Change in pH		Drug content %	
	4-8 °C	50 ± 2°C	4-8 °C	50 ± 2°C
0	5.21	5.21	98.0	98.0
7	5.21	5.21	97.8	97.6
14	5.21	5.30	97.7	96.4
21	5.21	5.30	96.3	95.3
30	5.21	5.30	69.2	94.8

6.11 Analysis of Release mechanism of optimized formulation by kinetic model

The drug release profile was also evaluated for 'goodness-of-fit' into various mathematical model equations such as zero order, first order, Higuchi matrix, Korsmeyer-Peppas and Hixson-Crowell cube root equation by using DD solver® tool. These kinetic models were used to understand the release mechanism of drug from the shampoo. The r^2 and k values of the model equation are shown in table 6.29. The model with r^2 value nearest to 1.000 was considered as the 'best-fit'model for the formulation. The maximum r^2 value was found to be for kosmeyer peppas model with n > 0.5 which indicates release following fickian diffusion and secondly for Hixson Crowell model. Therefore, Korsemeyer-Peppas model depicts the release as modified due to presence of lipid and surfactants for diffusion of drug and the second model represents dissolution rate limited drug release (Hina *et al.*, 2015)

Table 6.29

Various kinetic models of optimized shampoo (OPS)

S.No.	Zero Order	First Order	Higuchi model	Hixson Crowell	Korsmeyer Peppas
Itraconazole Shampoo	K= 1.14 r2= 0.962	K= 0.021 r2= 0.964	K= 9.19 r2= 0.957	K= 0.006 r2= 0.981	K= 3.46 r2= 0.996 n= 0.737
Iraz® Shampoo	K= 1.136 r2= 0.91	K= 0.022 r2= 0.981	K= 9.23 r2= 0.986	K= 0.006 r2= 0.983	K= 3.46 r2= 0.996 n= 0.737
*** K is slope except in first order where K = slope × 2.303**					

6.12 In vitro antifungal study

6.12.2 Zone of inhibition

The antifungal activity of Itraconazole from optimized SLNs shampoo was compared with ketocip® shampoo by measuring the zone of inhibition (Larson E, 1998) as shown in table 6.30 and Fig. 6.37. The obtained results for the optimized SLNs shampoo offered better antifungal potential by presenting the average inhibition zone measured to be 34.67 mm, whereas for Iraz® Shampoo, it was 29 mm in radius. These results are in agreement with the results obtained from the *in vitro* release study. This indicated good correlation between the chosen formulation and the *in vitro* antimicrobial susceptibility testing.

Table 6.30

The inhibition zones of gels of fluconazole

Formulation	Zone of Inhibition Diameter (mm)	
Iraz® Shampoo	31	29
Itraconazole Shampoo	33	34.67

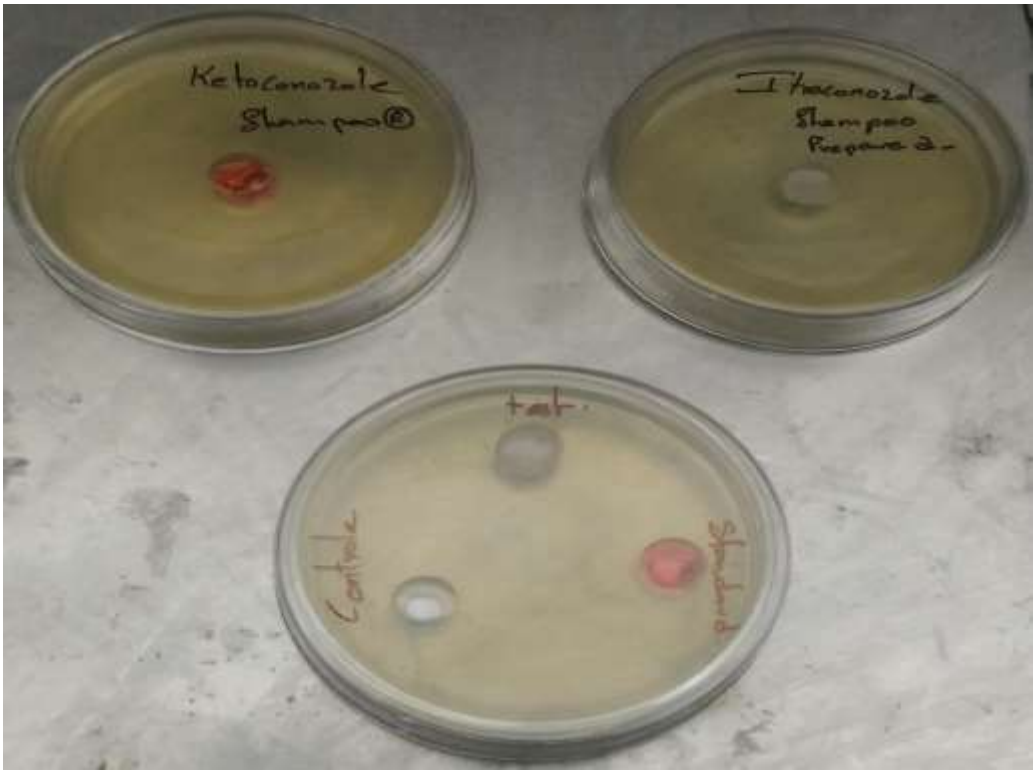


Fig. 6.35. Showing SDA media with cups (10 mm) with test, standard and control formulation together and individual in separate agar medium

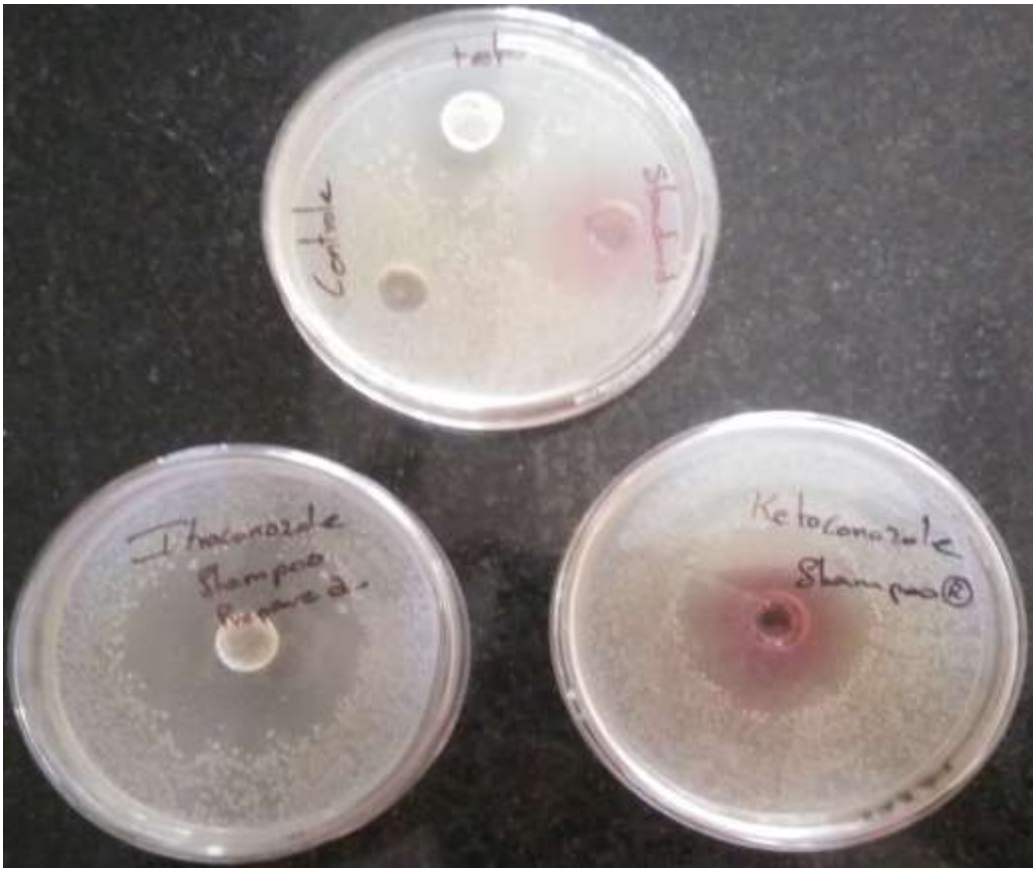


Fig. 6.36. Showing Zone of inhibition of control, standard and optimized formulation

CHAPTER 7

SUMMARY AND CONCLUSION

Summary and Conclusion

Ringworm, candidiasis and different types of leishmaniasis are a kind of fungal infections which are very common. Many people suffer from various fungal infections and take medication for treatment. So, to avoid the side effects of oral dosage form and to improve the efficacy and potency of conventional topical dosage form, novel SLNs shampoo system was developed.

Itraconazole was selected as a model drug. The characterization of Itraconazole was analyzed by melting point analysis and FTIR. The solubility analysis and partition coefficient was recorded to ensure the nature of drug. Analytical method of validation for Itraconazole in methanol was carried to establish a simple and reproducible analytical method for estimation of Itraconazole U.V spectrophotometrically. Prescreening studies were performed to decide the range and quantities of cholesterol, span 60, methanol and tween 80. The results from the prescreening study were implemented in design of expert software by using central composite design. Twenty eight formulations F_1 –F_{28} were prepared with varying amount of span 60, methanol, tween 80 and Itraconazole. The runs suggested by the software Design Expert® were prepared and were tested for two responses *i.e.* percentage entrapment efficiency and percentage permeability. This data was entered into Design Expert software and 47 formulations were suggested depending upon the ranges entered and the selected design i.e. CCD. The design was analyzed and the responses measured were entrapment efficiency and percent permeability. The five suggested optimized batches were selected. These were further validated. The validation was carried out by preparing the batches and observing the responses. The difference between the predicted and experimental value was recorded as the percent error which was within the range of ± 5%. The optimized nano particle formulation was studied for morphology by TEM, which ensured the formation of spherical shape. The zeta size analysis was carried which presented the nano size average range of 90.4 nm. The drug entrapment efficiency of the nano suspension dispersion was analyzed. The nano suspension dispersions were then, incorporated into shampoo. The optimized SLNs shampoo

was studied for various evaluation parameters such as pH determination, viscosity, foaming index, drug content, *in vitro* release, nano suspension dispersion stability and release kinetics. The drug retention after 90 min of study for SLNs shampoo was higher as compared to marketed antifungal shampoo. The *in vitro* antifungal activity of the SLNs shampoo was compared with that of marketed antifungal shampoo Iraz®, the *in vitro* antifungal activity of SLNs shampoo was found to be higher than the marketed antifungal shampoo.

From the different studies which were carried on nano suspension dispersion and SLNs shampoo, It could be concluded that the optimized SLNs shampoo presented promising results as a formulation to treat fungal infections topically on scalp and may be further studied to convert it into a commercial product.

FUTURE ASPECTS:

The present study has provided the information regarding the formulation development of SLNs shampoo of Itraconazole to enhance the efficiency of the shampoo to treat the fungal infections. The sincere efforts have been devoted to explore all the possible outcomes related to the development, validation and evaluation of the system. However, there is always a scope for a researcher to proceed further. The future aspects of the study involves: *Ex vivo* study of the SLNs shampoo to treat fungal infections along with various histopathalogical studies to ensure the safety profile of the developed system. The nano suspension system has emerged as a promising approach for the pharmaceutical as well as cosmeceuticals. Many researchers have focused their research for developing simple processing techniques to make the production of nano suspension more economical. With the advancement and researches carried nano suspension more system has emerged as a potential delivery system to serve as a suitable particle system for pharmaceuticals and cosmeceuticals in future.

CHAPTER 8

REFERENCES

Al-Jenoobi, FI., 2010. Effect of Itraconazole on the Pharmacokinetics of Diclofenac in Beagle Dogs. 78, 465–471.

Al-Sarra, IA., Alanazi, FK., Ahmed, SM., Bosela, AA., Alhamed, SS., Mowafy, HA., Neau, SH., 2010. Comparative study of itraconazole-cyclodextrin inclusion complex and its commercial product. 33, 1009-1017.

Antonio J. Almeida and Eliana Souto, Adv. Drug Delivery Rev., **59**, 478-490 (2007).

Biju, S.S., Talegaonkar, S., Mishra, P.R., Khar, R.K., 2006. Vesicular system: An overview. Indian J Pharm sci. 68, 141-153.

Beule, KD., 1996. Itraconazole: pharmacology, clinical experience and future development. 3, 175-181.

Buckner, SL., Ceesay, MM., Pagliuca, A., Morgan, PE., Flanagan, RJ., 2011. Measurement of posaconazole, itraconazole, and hydroxyitraconazole in plasma/serum by high-performance liquid chromatography with fluorescence detection. 33, 735-741.

Chen, W., Changyou, Z., Bing, G., Qinggang, M., Hao W., Weiyue, L., Huimin, H., 2011. Targeted brain delivery of itraconazole via RVG29 anchored nanoparticles. 228-234.

Chonn, A., Cullis, P.R., 1995. Recent advances in liposomal drug-delivery systems. Curr. Opin. Biotechnol. 6, 698-708.

Chudasama, A., Patel, V., Nivsarkar, M., Vasu, K., Shishoo, C., 2011. Investigation of microemulsion system for transdermal delivery of itraconazole. 1, 30-38.

Chudasama, A., Patel, V., Nivsarkar, M., Vasu, K., Shishoo, C., 2011. Investigation of microemulsion system for transdermal delivery of itraconazole. 2, 30-38.

Das, J., Majumdar, M., Chakraborty, U., Vivek, M., Gautam, M., Jayasree, N., 2011. Oral Itraconazole for the treatment of severe seborrhoeic dermatitis. 56, 515–516.

Ekambaram, P., Sathali, A.A.H., Priyanka, K., 2012. Solid Lipid Nanoparticles A Review.Scientific Reviews chemical Communications. 2, 80-102.

Engers, D., Teng, J., Jimenez-Novoa, J., Gent, P., Hossack, S., Campbell, C., Thomson, J., Ivanisevic, I., Templeton, A., Byrn, S., Newman, A., 2010. A solid-state approach to

enable early development compounds: selection and animal bioavailability studies of an itraconazole amorphous solid dispersion. 99, 3901-3922.

Gangwar, M., Singh, R., Goel, R.K., Nath,G., 2012. Recent advances in various emerging vesicular systems: An overview. Asian Pac J Trop Biomed. 4, 1176-1188.

Hayes, D., Ball, AM., Mansour, HM., Martin, CA., Flynn, JD., 2011. Fungal infection in heart-lung transplant recipients receiving single-agent prophylaxis with itraconazole. 6, 399-404.

Hire, N., Gudsoorkar, V., Bhise, K., Upasani, C., Nandgude, T., Dalvi., 2007. Microparticulate drug delivery system for topical administration of Itraconazole. 2, 83-88.

Hong, JY., Kim, JK., Song, YK., Park, JS., Kim, CK., 2006. A new self-emulsifying formulation of itraconazole with improved dissolution and oral absorption. 110, 332-338.

Indu Pal Kaur, Rohit Bhandari, Swati Bhandari and Kakkur. J. Cont. Rel., **127**, 97-109 (2008).

Joseph Robinson and Vincent H. L. Lee, Controlled Drug Delivery - Fundamentals and Applications, 2nd Edition, 4-33.

Jung, JY., Yoo, SD., Lee, SH., Kim, KH., Yoon, DS., Lee, KH., 1999. Enhanced solubility and dissolution rate of itraconazole by a solid dispersion technique. 187, 209-218.

Kapsi, SG., Ayres, JW., 2001. Processing factors in development of solid solution formulation of itraconazole for enhancement of drug dissolution and bioavailability. 229, 193-203.

Kazi, K.M., Mandal, A.S., Biswas, N., Guha, A., Chatterjee, S., Behera, M., Kuotsu, K., 2010. Niosome: A future of targeted drug delivery system. J Adv pharm Technol Res. 1, 374-380.

Lass, FC., 2011. Triazole antifungal agents in invasive fungal infections: a comparative review. 71, 2405-2419.

Li, DX., Park, YJ., Oh, DH., Joe, KH., Lee, JH., Yeo, WH., Yong, CS., Choi, HG., 2010. Development of an itraconazole-loaded gelatin microcapsule with enhanced oral bioavailability: physicochemical characterization and in-vivo evaluation. 62, 448-55.

REFERENCES

Linn, M., Collnot, EM., Djuric, D., Hempel, K., Fabian, E., Kolter, K., Lehr, CM., 2012. Soluplus® as an effective absorption enhancer of poorly soluble drugs in vitro and in vivo. 45, 336-43.

Melike Uner, Gulgun Yener, Int. J. Nanomedicine, **2(3)**, 289-300 (2007).

Miller, MA., DiNunzio, J., Matteucci, ME., Ludher, BS., Williams, RO., Johnston, KP., 2012. Flocculated amorphous itraconazole nanoparticles for enhanced in vitro supersaturation and in vivo bioavailability. 38, 557-570.

Miyake, K., Irie, T., Arima, H., Hirayama, F., Uekama, K., Hirano, M., Okamaoto, Y., 1999. Characterization of itraconazole/2-hydroxypropyl-beta-cyclodextrin inclusion complex in aqueous propylene glycol solution. 179, 237-245.

Mozafari, M. R., 41-50 (2006).

Mukherjee, S., Ray, S., Thakur, RS., 2009. Design and evaluation of itraconazole loaded solid lipid nanoparticulate system for improving the antifungal therapy. 2, 131-138.

Mukherjee, S., Ray, S., Thakur, R. S., 2009. Ind. J. Pharm. Sci., 349-358 .

Muller, RH., Mader, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery A Review of the state of the art. European Journal of Pharmaceutics and Biopharmaceutics. 50, 161-177.

Parikh, S.K., Ankit, D.P., Dr. Dave, J.B., Dr. Patel, C.N., Dr. Sen, D.J., 2011. Development and Validation of UV Spectrophotometric Method for Estimation of Itraconazole Bulk Drug and Pharmaceutical Formulation. International Journal of Drug Development & Research. 3, 324-328

Park, YJ., Xuan, JJ., Dong, HO., Balakrishnan, P., Ho-Joon Y., Woo HY., Lee, MK., Han, GC., Chul SY., 2010. Development of Novel Itraconazole-loaded Solid Dispersion without Crystalline Change with Improved Bioavailability. 33, 1217-1225.

Patel,V. R., Agarwal,Y.K., 2011. Nanosuspension: An approach to enhance solubility of drug. J Adv pharm Technol Res. 2, 81-87.

Prasad, RS., Sarath, KY., Manavalan, R., 2010. Preparation and characterization of Itraconazole solid dispersions for improved oral bioavailability. 2, 133-142.

Rainer, H., Muller., Karsten, M., Sven, G., 2000. European Journal of Pharmaceutics and Biopharmaceutics. **50**, 161-177.

REFERENCES

Shao, PL., Huang, LM., Hsueh, PR., 2007. Recent advances and challenges in the treatment of invasive fungal infections. 6, 487-495.

Shim, SY., Ji, CW., Sah, H., Park, ES., Lee, BJ., 2006. Characterization of itraconazole semisolid dosage forms prepared by hot melt technique. 11, 1055-1060.

S. P. Vyas and R. K. Khar, Controlled Drug Delivery - Concepts and Advances, First Edition, Vallabh Prakashan (2002) pp. 38-50.

Tang, J., Wei, H., Liu, H., Ji, H., Dong, D., Zhu, D., Wu, L., 2010. Pharmacokinetics and biodistribution of itraconazole in rats and mice following intravenous administration in a novel liposome formulation. 17, 223-230.

Tao, T., Zhao, Y., Jinjin, W., Beiyi, Z., 2009. Preparation and evaluation of itraconazole dihydrochloride for the solubility and dissolution rate enhancement. 367, 109-114.

Wang, J., Huang, G., 2011. Preparation of Itraconazole-loaded liposomes coated by carboxymethyl chitosan and its pharmacokinetics and tissue distribution. 8, 631-638.

Wolfgang, M., Karsten, M., Adv. Drug. Deliv. Rev., **47**, 165-196 (2001).

Yadav, N., khatak, S., Sara, U.V.S., 2013. Solid Lipid Nanoparticles A Review. International Journal of Applied Pharmaceutics. 5, 8-9.

Yang, W., Tam, J., Miller, DA., Zhou, J., McConville, JT., Johnston, KP., Williams, RO., 2008. High bioavailability from nebulized itraconazole nanoparticle dispersions with biocompatible stabilizers. 361, 177-188.

Ahmed, S., Mustaan, N., Sheraz, M.A., Nabi, S.A.A. un, Ahmad, I., 2015. Validation of a UV Spectrometric Method for the Assay of Tolfenamic Acid in Organic Solvents. J. Pharm. 2015, 1–8. doi:10.1155/2015/216249

Andhale, V.A., Patil, P.R., Dhas, A.U., Chauhan, P.D., Desai, S. V., 2016. Liposome: An emerging tool in drug carrier system. Int. J. Pharm. Technol. 8, 10982–11011. doi:10.7897/2321

Aparna, C., Srinivas, P., modyala, D., 2014. Formulation , Evaluation and Characterization of Itraconazole Lozenges. IOSR J. Pharm. Biol. Sci. 9, 86–94. doi:10.9790/3008-09318694

Bagavatula, H., Lankalapalli, S., Vinai, V.S., Tenneti, K., 2014. Comparative Studies on Solubility and Dissolution Enhancement of Different Itraconazole Salts and Their Complexes. Adv. Pharmacol. Pharm. 2, 85–95. doi:10.13189/app.2014.020601

Deveda, P., Jain, A., Vyas, N., Khambete, H., Jain, S., 2010. Gellified emulsion for sustain delivery of itraconazole for topical fungal diseases. Int. J. Pharm. Pharm. Sci. 2, 104–112.

REFERENCES

Kumar, N., Goindi, S., Saini, B., Bansal, G., 2014. Thermal characterization and compatibility studies of itraconazole and excipients for development of solid lipid nanoparticles. J. Therm. Anal. Calorim. 115, 2375–2383. doi:10.1007/s10973-013-3237-6

M, T.R., J, V.R., Y, S.R., T, H.K., 2015. Development and Validation of RP-HPLC Method for the Estimation of Voriconazole in Bulk and Pharmaceutical Dosage Form. Int. J. Pharm. Sci. Rev. Res. 31, 221–225. doi:10.7598/cst2014.897

Mohamed, M.S., Eid, A.M.M., Elgadir, M.A., Mahdy, M.A., 2013. Preparation and release charcteristics of Itraconazole poymeric films for topical application. Int. J. Pharm. Pharm. Sci. 5, 167–170.

Mukherjee, S., Ray, S., Thakur, R.S., 2009. Design and evaluation of Itraconazole loaded solid lipid nanoparticulate system for improving the antifungal therapy. Pak. J. Pharm. Sci. 22, 131–138. doi:s

Nakarani, M., Misra, A.K., Patel, J.K., Vaghani, S.S., 2010. Itraconazole Nanosuspension Meant for Oral Use : Development of Formulation , Characterization and In Vitro Comparison with Some. Daru 2, 162–171.

Nandgude, T.D., Dalvi, H., 2007. Asia n Jou r n a l of Pha rm aceut ics Or igin a l Re se a r ch Ar t icle s MICROPARTICULATE DRUG DELIVERY SYSTEM FOR TOPICAL ADMINISTRATION Asia n Jou r n a l of Pha rm aceut ics 1.

Parikh, S.K., Patel, A.D., Dave, J.B., Patel, C.N., Sen, D.J., 2011. Development and validation of UV spectrophotometric mathod for estimation of itraconazole bulk drug and pharmaceutical formulation. Int. J. Drug Dev. Res. 3, 176–179.

Patel, J.N., Rathod, D.M., Patel, N.A., Modasiya, M.K., 2012. I NTERNATIONAL J OURNAL OF P HARMACY & L IFE S CIENCES 3, 1459–1469.

Pawar, P.K., Majumdar, D.K., 2007. In vitro permeation characteristics of moxifloxacin from oil drops through excised goat, sheep, buffalo and rabbit corneas. Pharmazie 62, 853–857. doi:10.1691/ph.2007.11.7055

Prasad, R.S., Yandrapu, S.K., Manavalan, R., 2010. Preparation and characterization of itraconazole solid dispersions for improved oral bioavailability. Int. J. ChemTech Res. 2, 133–142.

Prasuna Sundari, P., Vijaya Shanthi, V., 2013. A Review: Analytical Methods for Determination of Itraconazole in Pharmaceutical and Biological samples 1, 21–24.

Reddy, B., Venkateswara, S.R.I., Of, G., 2016. FORMULATION AND IN VITRO ASSESSMENT OF ITRACONAZOLE LOADED SOLID LIPID NANOPARTICLES FOR TOPICAL DELIVERY Advanced Biomedical & Pharmaceutical Research FORMULATION AND IN VITRO ASSESSMENT OF ITRACONAZOLE LOADED.

REFERENCES

Sampathi, S., Ajimera, T., Kuchana, V., 2014. Preparation and Evaluation of Liposome Entrapped Hydrogel Complex Systems of Itraconazole for Enhanced Transdermal Permeation. J. Pharm. Sci. Innov. 3, 25–29. doi:10.7897/2277-4572.031100

Sawant, A.A., Mohite, S.K., 2015. Formulation and Evaluation of Itraconazole Emulgel for Topical Drug Delivery. Asian J. Pharm. Technol. 5, 91. doi:10.5958/2231-5713.2015.00014.8

Wagh, V.D., Deshmukh, O.J., 2012. Itraconazole Niosomes Drug Delivery System and Its Antimycotic Activity against Candida albicans. ISRN Pharm. 2012, 653465. doi:10.5402/2012/653465

Yoganandam, Prakash G; Gopal, V; Kaviarasan, L., 2012. Pharmacy Review & Research 2, 93–96.

Pallavi V. Pople and Kamalinder K. Singh, AAPS Pharm. Sci. Tech., **7(4)**, 91 (2006).

Gande Suredh, Kopparam Manjunath, Vobalaboina Venkateswarlu and Vemula Styanarayana, AAPS Pharm. Sci. Tech., **8(1)**, 24 (2007).

Elnaggar YSR, El-Massik MA, Abdallah OY. Fabrication, appraisal, and transdermal permeation of sildenafil citrate-loaded nanostructured lipid carriers versus solid lipid nanoparticles. International Journal of Nanomedicine 2011; 6 :3195–3205.

Bhaskar K, Anbu J, Ravichandiran V, Venkateswarlu VRao YM. Lipid nanoparticles for transdermal delivery of flurbiprofen: formulation, in vitro, ex vivo and in vivo studies. Lipids in Health and Disease2009; 8 Supply 6.

Salador,A.,andChisvert,A.:Analysisofcosmeticproducts,Elsevier,NewYork,2006.

Thatipamula RP., Palem CR., Gannu R., Mudragada S.,Yamsani MR. formulation and in vitro characterization of domperidone laded solid lipid nanopaticle and nanostructured lipid carriers:jouranls tumesac ir. 2011;(19):23-32

Sarkar B, Patel R, Bhadoriya U .Antimicrobial Activity of Some Novel Pyrazoline Derivatives. J Advanced Pharm Edu & Res .2011; 243-250

Annette Zur Mehlen, Cora Schwarz and Wolfgang Mehnart, Eur. J. Pharm. Biopharm., **45**, 149-155 (1998).

Dong Zhi Hou, Chang Sheng Xie, Kaijn Huang and Chang Hong Zhu, Biomaterials, **24**, 1781-1785 (2003).

Wei Liu, Meling Hu, Wehsuang Liu and Chengbin Xue, Huibi Xu, Int. J. Pharm., **364**, 141-146 (2008).

K. Vivek, Harivardhan Reddy and Ramachandra S. R. Murthy, AAPS Pharm. Sci. Tech., **8(4)**, Article 83 (2007).

REFERENCES

Biswajit Basu, Kevin Garala, Ravi Bhalodia and Bhavik Joshi, Kuldeep Mehta, J. Pharm. Res., **3(1),** 84-92 (2010).

Shah M, Pathak K. Development and Statistical Optimization of Solid Lipid Nanoparticles of Simvastatin by Using 23 Full-Factorial Design. AAPS PharmSciTech2010; 11 Suppl 2 : 489-496.

Jawahar N, Gowthamarajan K, Meyyanathan SN, Sood S. Brain delivery by Solid Lipid Nanoparticles for CNS drugs. International journal of Pharmaceutical Research and Development2011; 3 Supply 4 :206-216.

S. P. Vyas and R. K. Khar, Controlled Drug Delivery - Concepts and Advances, First Edition, Vallabh Prakashan (2002) pp. 38-50.

Chakraborty S, Shukla D, Mishra B, Singh S. Lipid – An emerging platform for oral delivery of drugs with poor bioavailability. European Journal of Pharmaceutics and Biopharmaceutics 2009; 73 :1–15.

Helgason T, Awad TS, Kristbergsson K, McClements DJ, Weiss J. Effect of surfactant surface coverage on formation of solid lipid nanoparticles (SLN). Journal of Colloid and Interface Science2009; 334 :75–81.

Maravajhala V, Papishetty S, Bandlapalli S. Nanotechnology in Development of Drug Delivery System. International Journal of Pharmaceutical Science and Research2011; 3 Suppl 1 :84-96.

Loxley A. Solid Lipid Nanoparticles for the Delivery of Pharmaceutical Actives. Drug Delivery Technology2009; 9 Suppl 8 :32.

Gonnet M, Lethuaut L, Boury F. New trends in encapsulation of liposoluble vitamins. Journal of Controlled Release2010; 146 :276–290.

Shuyu Xie, Luyan Zhu, Zhao Dong and Yan Wang, Colloids and Surfaces B: Biointerfaces, **83**, 382-387 (2011).

Mehnert W, Mader K. Solid lipid nanoparticles-Production, characterization and applications. Advanced Drug Delivery Reviews 2001; 47 :165–196.

Alaa Eldeen B. Yassin, Md Khalid Anwer and Ibrahim A. Alsarra, Int. J. Medical Sci., **7(6)**, 398-408 (2010).

T. Helgason, T. S. Awas, K. Kristbergsson and J. Weiss, J. Colloid, Interface Science, **334**, 75-81(2009).

REFERENCES

Robhash Kusam Subedi, Keon Wook Kang and Hoo-Kyun Choi, Eur. J. Pharm. Sci., **37**, 508-513(2009).

Behzad Sharif Makhmal Zadeh and Fakher Rahim, Int. J. Res. Pharm. Sci., **1(4)**, 466-472 (2010).

Yingchao Li. Lei Dong, Xinming Chang and Hui Xue, Int. J. Biological Macromolecules, **38**, 296-299 (2006).

Qing Zhi Lu, Aihua Yu, Yanwei Xi and Houli Li, Zhimei Song, Jing Cui and Fengliang Cao, Guangxi Zhai, Int. J. Pharm., 372, 191 – 198 (2009).

Alessandro Bargoni, Roberto Cavalla, Otto Caputo and M. R Gasco, Pharm. Res., **15(5)**, 745-750 (1998).

Eldem T, Speiser P, Hincal A. Optimization of spray-dried and congealed lipid microparticles and characterization of their surface morphology by scanning electron microscopy. Pharm Res 1991;8:47-54.

Speiser P. Lipidnanopellets als Tragersystem fur Arzneimittel zur peroralem Anwendung. European Patent No. EP 0167825; 1990.

Meyer E, Heinzelmann H. Scanning force microscopy. In: Wiesendanger R, Guntherodt HJ. Editors. Scanning tunneling microscopy II, Surface science. New York: Springer Verlag; 1992. p. 99-149.

Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J Control Release 1999;59:299-307.

Zur Muhlen A, Mehnert W. Drug release and release mechanism of prednisolone loaded solid lipid nanoparticles. Pharmazie 1998;53:552-5.

Drake B, Prater CB, Weisenhorn AL, Gould SAC, Albrecht TR, Quate CF, *et al.* Imaging crystals polymers and process in water with the AFM. Science 1989;243:1586-9.

Jenning V, Gysler A, Schafer-Korting M, Gohla SH. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. Eur J Pharm Biopharm 2000;49:211-8.

Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J Control Release 1999;59:299-307.

REFERENCES

Wong HL, Rauth AM, Bendayan R. A new polymer-lipid hybrid nanoparticle system increases cytotoxicity of doxorubicin against multidrug-resistant human breast cancer cells. Pharm Res 2006;23:1574-85.

Lai F, Wissing SA, Muller RH, Fadda AM. Artemisia arborescens L essential oil-loaded solid lipid nanoparticles for potential agriculture application: preparation and characterization. AAPS Pharm Sci Tech 2006;7(1):E2

Pedersen N, Hansen S, Heydenreich AV, Kristensen HG, Poulsen HS. Solid lipid nanoparticles can effectively bind DNA, streptavidin and biotinylated ligands. Eur J Pharm Biopharm 2006;62:155-62.

Rudolph C, Schillinger U, Ortiz A, Tabatt K, Plank C, Muller RH, *et al.* Application of novel Solid lipid nanoparticles (SLN)- gene vector formulations based on a diametric HIV-1 VAT peptide in vitro and in vivo. Pharmaceutic Res 2004;21(9):1662-9.

DongZhi H, ChangSheng X, Kaijin H, ChangHong Z. The production and Characteristics of solid lipid nanoparticles (SLNs). Biomaterials 2003; 24:1781-1785.

Rainer HM, Karsten M, Sven G. Solid lipid nanoparticles (SLN) for controlled drug delivery- a review of the state of the art. Eur J Pharm Biopharm 2000; 50:161-177

Muller RH, Radtka M, Wissing SA. Solid lipid nanoparticle (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Del Rev 2002; 54(1): S131-S155.

Wolfgang M, Karsten M. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Del Rev 2001; 47:165-196.

Wissing SA, Kayser O, Muller RH. Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Del Rev 2004; 56:1257–1272.

Anh Nguyen, V´eronique Marsaud, C´eline Bouclier, Siden Top, Anne Vessieres, Pascal Pigeon, Ruxandra Gref, Philippe Legrand, G´erard Jaouen, Jack-Michel Renoir. Nanoparticles loaded with ferrocenyl tamoxifen derivatives for breast cancer treatment. Int J Pharm 2007; 06 (033):1-27.

Giacomo Fontana, Laura Maniscalco, Domenico Schillaci, Gennara Cavallaro, Gaetano Giammona. Solid Lipid Nanoparticles Containing Tamoxifen Characterization and *In Vitro* Antitumoral Activity. Drug delivery 2005; 12(6):385-92.

REFERENCES

Hae Sun Suh, Myoengsin Choi, Kyu Won Han, So Min Park and Kil-Soo Kim. Preparation and Evaluation of Solid Lipid Nanoparticles (SLNs) containing 5-Fluorouracil and Its Derivative. J. Kor. Pharm. Sci 2005; 35(3):143-150.

Andreas Illing, Tobias Unruh. Investigation on the flow behavior of dispersions of solid triglyceride nanoparticles. Int J Pharm 2004; 284: 123–31.

Vobalaboina Venkateswarlu and Kopparam Manjunath. Preparation, characterization and *in vitro* release kinetics of clozapine solid lipid nanoparticles. J Control Release 2004; 95(3, 24):627-38.

Michele Trotta, Francesca Debernardi, Otto Caputo. Preparation of solid lipid nanoparticles by a solvent emulsification–diffusion technique. Int J Pharm 2003; 257:153–60.

Bin Lu, Su-Bin Xiong, Hong Yang, Xiao-Dong Yin, Ruo-Bing Chao. Solid lipid nanoparticles of mitoxantrone for local injection against breast cancer and its lymph node metastases. Eur J of Pharm Sci 2006; 2 8: 86-95.

Waree Tiyaboonchai, Watcharaphorn Tungpradit, Pinyupa Plianbangchang. Formulation and characterization of curcuminoids loaded solid lipid nanoparticles. Int J Pharm 2007; 337:299–306.

Elena Ugazio, Roberta Cavalli, Maria Rosa Gasco. Incorporation of cyclosporine- A in solid lipid nanoparticles (SLN). Int J Pharm 2002; 241: 341–344.

Hu FQ, Yuan H, Zhang HH, Fang M. Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. Int J Pharm 2002; 239: 121–128.

Alix Dubes, Hélène Parrot Lopez, Wassim Abdelwahed, Ghania Degobert, Hatem Fessi, Patrick Shahgaldian and Anthony W Coleman. Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins. Eur J Pharm Biopharm 2003; 55(3):279-282.

Ji-Yao Zhang, Zhi-Gang Shen, Jie Zhong, Ting-Ting Hu, Jian-Feng Chen, Zhong-Qing Ma, Jimmy Yun. Preparation of amorphous cefuroxime axetil nanoparticles by controlled nanoprecipitation method without surfactants. Int J Pharm 2006; 323:153-160.

Pallavi V Pople and Kamalinder K Singh. Development and evaluation of topical formulation containing solid lipid nanoparticles of vitamin-A. AAPS Pharm Sci Tech 2006; 7 (4):E1-E7.

REFERENCES

Kumar VV, Chandrasekar D, Ramakrishna S, Kishan V, Rao YM, Diwan PV. Development and evaluation of nitrendipine loaded solid lipid nanoparticles: influence of wax and glyceride lipids on plasma pharmacokinetics. Int J Pharm 2006; 11(004):01-32.

Hina Kouser Shaikh., R. V. Kshirsagar., S. G. Patil., 2015 Mathematical Models For Drug Release Characterization: A Review. World Journal Of Pharmacy And Pharmaceutical Sciences. 4, 324-338.