“FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES LOADED WITH ORLISTAT FOR ORAL DRUG DELIVERY”

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

MASTER OF PHARMACY
IN

PHARMACEUTICS
By

Keerti Bhardwaj
(Reg. No. 11507540)

Under the guidance of

Ms. Ruchi Sharma
(Assistant Professor)

School of Pharmaceutical Sciences

Lovely Faculty of Applied Medical Sciences

Lovely Professional University

PUNJAB-144411

May, 2017

Dedicated to my parents and the Almighty

TOPIC APPROVAL PERFORMA

LIT (Pharmacy)/Department of Pharmaceutical Sciences

Program : P570-NN1::M.Pharm. (Pharmaceutics)

COURSE CODE : PHS514 **REGULAR/BACKLOG :** Regular **GROUP NUMBER :** PHRRGD0043

Supervisor Name : Ruchi Sharma **UID :** 19260 **Designation :** Assistant Professor

Qualification : _____ **Research Experience :** _____

SR.NO.	NAME OF STUDENT	REGISTRATION NO	BATCH	SECTION	CONTACT NUMBER
1	Keerti Bhardwaj	11507540	2015	Y1507	9780410544

SPECIALIZATION AREA : Pharmaceutics **Supervisor Signature:** _____

PROPOSED TOPIC : Formulation and Evaluation of nano-particles of Orlistat to improve the bioavailability for treatment of obesity

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
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	6.00
5	Social Applicability: Project work intends to solve a practical problem.	6.00
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	6.50

PAC Committee Members		
PAC Member 1 Name: Dr. Amit Mittal	UID: 13145	Recommended (Y/N): NA
PAC Member 2 Name: Saurabh Singh	UID: 12208	Recommended (Y/N): NA
PAC Member 3 Name: Dr. S. Tamilvanan	UID: 16391	Recommended (Y/N): Yes
PAC Member 4 Name: Dr. Navneet Khurana	UID: 18252	Recommended (Y/N): NA
DAA Nominee Name: Dr. Sazal Patyar	UID: 17050	Recommended (Y/N): Yes

Final Topic Approved by PAC: **Formulation and Evaluation of nano-particles of Orlistat to improve the bioavailability for treatment of obesity**

Overall Remarks: Approved

PAC CHAIRPERSON Name: 11045::Dr. Monica Gulati **Approval Date:** 02 Dec 2016

ACKNOWLEDGEMENT

First and foremost I submit my thesis at the feet of ALMIGHTY for all of his blessings.

It is my honor that I am able to express my utmost gratitude with a sense of elation and respect to my project guide **Ms. Ruchi Sharma,** Assistant Professor, Lovely School of Pharmaceutical Sciences, Lovely Professional University, for her constant guidance, supervision, and motivation in undertaking and completing my project work. My sincere thanks for her sumptuous suggestions and constructive criticisms to embellish my study. It is with great privilege and pleasure that I submit this dissertation work as her student. I would like to thank my parents who have always been with me. It was due to my parents' dream, ambition, and sacrifice that I gained so much drive and an ability to tackle all the challenges during this research work. Today with humility, bowed head, misty eyes and folded hands, I am incapable of expressing my innermost thoughts into words for my family. I would like to express my heartiest thankfulness to my beloved family, my father **Mr. Satish Kumar Bhardwaj,** my mother **Mrs. Saroj Bhardwaj** and my brother **Mr. Varun Bhardwaj** as without their love and support it would not have been possible to complete the task.

I am highly indebted to **Dr. Monica Gulati,** Sr. Dean, Lovely School of Pharmaceutical Sciences, Lovely Professional University, whose continuous supervision helped me a lot to surmount many problems during the project. Acknowledgment can't be completed without expressing gratitude to our worthy chancellor **Mr. Ashok Mittal** who provided the facilities, equipment, and faculty without which, I would not have been able to reach my destination. Thanks are incomplete without naming who always spared their precious time and helped me out whenever required.

I express my gratitude to **all other faculty members** of the Lovely School of Pharmaceutical Sciences, for their encouragement and concern.

I express my heartful thanks to **Mr. Gopal Krishan** and **Mr. Harish** for providing me the laboratory equipment and chemicals for my project completion.

My sincere thanks to **Non-Teaching Staff,** and **Classmates** for their help during the work in the laboratory.

I take this opportunity to express my love, affection, and gratitude towards my friends: **Ms. Swati Patial, Ms. Rhythm Bassi, Ms. Pooja Sharma, Mr. Nuni Sagar, Mr. Yusuf Nawaz Khan** and **Mr. Deepak Ghai** for their cooperation and solid support during my project work.

Words will fall short to express my feelings for my Papa. Education commences at the mother's knees, My Mumma is the soul of my energy. Her blessings have lead me towards achieving my goals.

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

Date: 13/05/2017
Place: Jalandhar

Keerti Bhardwaj

Statement by the candidate

This is to submit that this written submission in my thesis entitled "**Formulation and evaluation of solid lipid nanoparticles loaded with Orlistat for oral drug delivery**" 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.

Patents related to API, process, product, method and equipment, if any, have been examined to ensure non-infringing approach to the existing patents.

This thesis encompasses the information generated by me based on experimental work carried out in the Lovely Professional University, Phagwara (Punjab). I assure and hold full responsibility for its genuineness.

Ms. Keerti Bhardwaj
Reg. No. 11507540

Forwarded Through

Ms. Ruchi Sharma
Assistant Professor
Domain of Pharmaceutics
School of Pharmaceutical Sciences
LFAMS, LPU

Certificate by Supervisor

The work described in this thesis entitled **"Formulation and evaluation of solid lipid nanoparticles loaded with Orlistat for oral drug delivery"** has been carried out by Ms. Keerti Bhardwaj under Ms. Ruchi Sharma's supervision. I certify that this is her bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Date: 13/05/2017

Place: Jalandhar

Ms. Ruchi Sharma
Assistant Professor
Domain of Pharmaceutics
School of Pharmaceutical Sciences
LFAMS, LPU
Research Supervisor

Certificate by School

This is certified that the work described in this project report entitled **"Formulation and evaluation of solid lipid nanoparticles loaded with Orlistat for oral drug delivery"** has been carried out by Ms. Keerti Bhardwaj at the School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

Dr. S. Tamilvanan
Professor
COD, Domain of Pharmaceutics
School of Pharmaceutical Sciences
LFAMS, LPU
Punjab, 144411
Head of Domain

Dr. Monica Gulati
Senior Dean
School of Pharmaceutical Sciences
LFAMS, LPU
Punjab, 144411
Head of School

ABSTRACT

The drug profile with poor solubility, instability, and poor bioavailability limits the physiochemical and pharmacokinetic performance of the drug molecule. Orlistat the drug of interest belongs to the category of anti-obesic agent which shows hydrophobic character and is being used in the study extensively in treatment and management of obesity. The drug inhibits pancreatic lipase enzyme secreted in stomach. Thus, it has to be administered orally for most effective results. But, the major dispute is related to its side is poor absorption when administered orally, which on the other flip hammer its effectiveness in management of anti-obesic therapy. Therefore, in the present study a multidisciplinary approach has been carried out in formulating the Solid lipid nanoparticles for oral delivery of Orlistat in the form of capsules to counteract the pre-existing setbacks in oral administration of the drug. The objective behind development of SLNs was due to numerous reporting through researchers related to its effecting potential of delivering lipophilic drugs through oral route which helps in bioavailability enhancement. The approach is empowered by articulating from conventional to lipid-based nano-carrier formulation and in addition, specific amendment in the functional components. It has been proposed through work that Orlistat loaded nano particle will work onto overcome the complications in an effective way. Therefore, the presenting work highlights the improved delivery of Orlistat through oral route providing better oral absorption in same amount of drug. The enhanced solubility and therefore the bioavailability results into dose reduction which also lowers the risk of side effects due to drug toxicity or due to higher doses.

Keywords: Orlistat, Solid lipid nanoparticles, Obesity, Anti-obesic, solubility, Oral drug delivery system

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
AUC	Area under curve
$^{\circ}C$	Degree Celsius
DCM	Dichloromethane
DLS	Differential Scanning Calorimetry
DSC	Dynamic Light Scattering
FT-IR	Fourier Transform Infrared spectroscopy
GI	Gastro-intestinal tract
GMS	Glyceryl monostearate
HPH	High Pressure Homegenisation
Kg	Kilogram
µg	Microgram
mg	Miligram
MUPS	Multi-Unit Pellet System
nm	Nanometer
O/W	Oil in water
PCS	Photon correlation spectroscopy
PEG	Polyethylene Glycol
PVA	Polyvinyl Alcohol
PXRD	Powder X-Ray Diffraction
SEDDS	Scanning Electron Microscopy
SEM	Self-Emulsifying Drug Delivery System
SLNs	Self-Microemulsifying Drug Delivery System
SMEDDS	Solid Lipid Nanoparticles
TEM	Transmission Electron Microscopy
W/O	Water in mineral oil in water double emulsion
w/o/w	Water in oil

CHAPTER 1

INTRODUCTION

1.1 Drug Delivery Systems

The drugs having problems related to their solubility and bioavailability are intended to be delivered through any novel delivery system to overcome the shortcomings and get the desired therapeutic effect on the body. The various techniques involved in enhancing the properties of drug involve precipitation, micronization, nanonization, use of surfactants or drug coating [Scheffel et al, 1970].

Other than these conventional methods, active attempts are being made to upgrade the drug efficacy by encapsulating the drug into suitable nano carriers as drug delivery systems. The efficacious implementation of nanoparticles as drug delivery system depends on upon various factors such as penetration capacity of the system through a number of physiological as well as anatomical barriers, the sustained release of their constituents and their ability to remain stabilized in nanometer size range also. However, the shortage of the variety of safe polymers, which could get the regulatory approval along with the high cost of the available polymers have made it more difficult for the application in nanoparticles formulation for clinical medicines [Lin et al, 2017]..

To conquer these limitations, lipid has been used in place of polymers to act as a delivery system, especially in case of lipophilic active constituents and these lipid-based nanoparticles are known as the Solid-Lipid Nanoparticles (SLNs) which are drawing more and more attention of the researchers [Jumaa et al, 2000].

The lipid matrix made in the SLNs are prepared by the physiologically tolerated fat content which results in the decrease in the potential risk of chronic or acute toxicity which can occur in case of polymeric nanoparticles [Menhert et al, 2001]. Therefore, it can be concluded that solid-lipid nanoparticles have the combined advantages of various delivery systems such as the low toxicity of liposomes and fatty emulsions and are capable of providing sustained release occurred because of the solid matrix just like the polymeric nanoparticles and can exhibit targeted drug delivery if administered parenterally [Zara et al, 1999; Yang et al, 1999].

The SLNs are considered to be the favourable systems of drug delivery in advanced era of submicron-sized emulsions of lipid in which the solid lipid is being used in place of the

liquid lipid (oil). These solid-lipid nanoparticles exhibit several properties like nano ranged size, larger surface area, increased drug loading and the interfacial interaction of phases. These systems have the capability to enhance the therapeutic performance of the pharmaceutically active materials [Cavalli et al, 1993].

1.2 Solid lipid nanoparticles for oral delivery

The lipid nanoparticles made with the solid matrix have emerged as an efficient carrier of drug for the enhancement of oral bioavailability and GI absorption of many poorly soluble drugs, chiefly the lipophilic ones. This system can also be used for the sustained release and are being considered and studied for their abilities to deliver the drug orally [Radtke et al, 2005]. The lipids chosen for the nanoparticles preparations should be biocompatible, biodegradable as well as physiological which minimizes the risk of toxicity associated with the polymeric nanoparticles. Along with that, the solid matrix results in the enhanced stability of the formulation as compared to other nano-carrier liquid preparations [Pouton et al, 2006]. Such nanoparticles preparations can be carried out using various techniques and it is easy to move up from lab scale production to industrial scale production during the process. It was observed that the solid lipid nanoparticles provide improved drug entrapment efficiency as well as bioavailability and oral absorption were also enhanced in the oral administration [Shidhaye et al, 2008]. Fig 1.1 shows the various pathways which could be followed by SLNs to reach into systemic circulation via oral route.

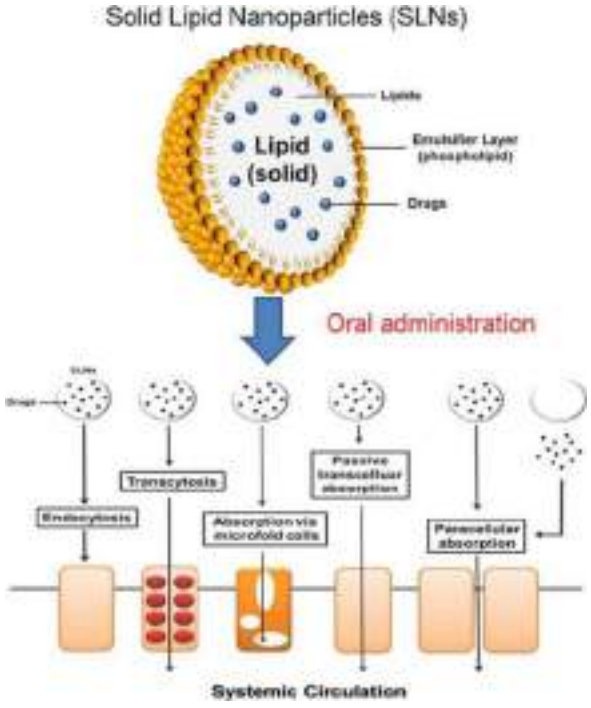


Fig 1.1 Solid lipid nanoparticles reaching systemic circulation via oral route [Lin et al, 2017]

Due to several combinations of lipids and their morphologies, the chemical and physical characteristics of these lipid-matrix based systems can get very complicated. The drug solubility is dependent upon their morphologies, the morphology interconversions with respect to time and their chemical structure along with the digestion of lipids all should be observed and kept in mind to get the desired results [Bummer et al, 2004].

The perks of lipid-based formulations cover:

→ The improved GI absorption and reduced instability of the lipophilic, poorly soluble drugs.

→ Feasible reduction or withdrawal of several processing operations and steps like selection of salt, determination of a steady crystalline form of the API, taste-making, coating and tedious process of clean-ups while manufacturing any cytotoxic or very potent drug products.

→ Reduced chances of food-drug interactions.

→ Comparative ease in manufacturing due to the use of easily available tools and equipments [Jannin et al, 2008].

The *in-vivo* outcome of SLNs depends primarily on the route of administration and the process of distribution i.e. the biological material adsorbing on the surface of particles and the SLN constituents desorbing in the biological atmosphere. Solid lipid nanoparticles consist of physiological lipids or related waxes which helps in the transportation pathways and the metabolism process to be carried out with more ease and hence assure the *in-vivo* journey of the carrier to a greater extent [Yang et al, 1999]. The process of lipid digestion in the body is shown in Fig. 1.2.

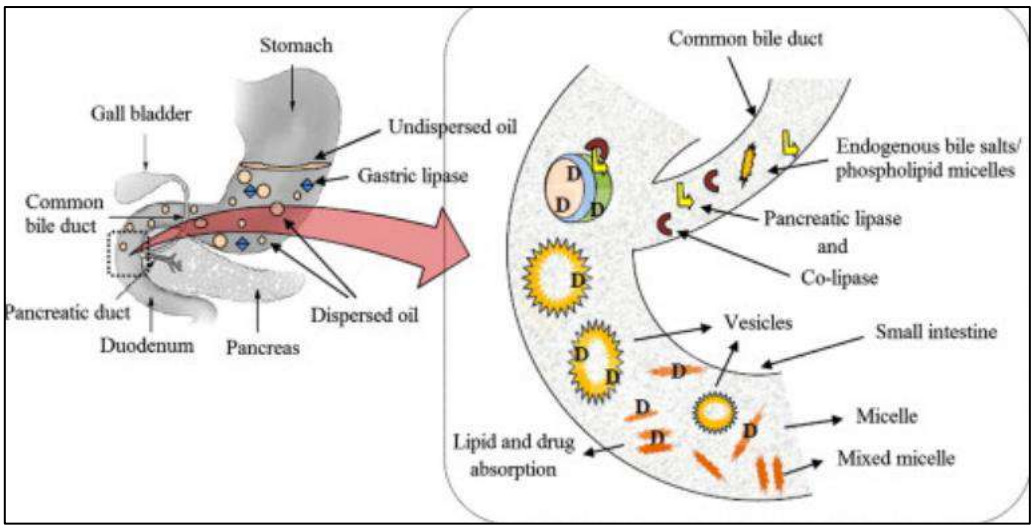


Fig 1.2 Digestion of lipid and process of drug solubilisation in small intestine [Kalepu et al, 2013]

Lipases are the most significant enzymes for the degradation of solid lipid nanoparticles and are present in many tissues and organs. Lipases produce free fatty acids and fractional glycerides by splitting the ester linkage. They need to get activated by an oil or water interface which leads to the opening of the catalytic centre. The *in-vivo* studies show that SLNs with different compositions show different velocities of degradation by the pancreatic lipase enzyme [Yang et al, 1999].

Every oral route solid lipid nanoparticle formulation either involves any aqueous dispersion or they are loaded in any conventional dosage form such as capsules, tablets or pellets. The environment of stomach supports the aggregation of particles because of the high ionic strength and acidity. It is quite possible that the food present in the stomach will have a great effect on the performance of the SLNs, although no such experimental proof or data have been found yet as per our knowledge [Mukherjee et al, 2009].

1.3 Problems related to Orlistat

Orlistat belongs to BCS class II which means it has low solubility and high permeability factors. Being a poorly water-soluble drug, the GI absorption and bioavailability of Orlistat get compromised. It is a waxy, sticky and fluffy compound with a remarkably low melting point of 46 °C which makes it susceptible to instability.

The formulation gets affected by the physicochemical properties of various excipients as well as the active pharmaceutical ingredient used in the preparation. Because of low melting point, the preparation of traditional dosage forms like capsules and tablets encounters troubles related to sticking and picking while compressing the tablets or while encapsulation. Moreover, considering the nature of the drug, it may undergo both thermal degradation and hydrolytic degradation.

The formulation processes may include various unit operations like sieving, slugging, milling, dry or wet granulation, encapsulation etc. producing mechanical energy which can be imparted into the processing material and it may lead to inactivation, melting or deformulation of the drug or intended drug product. The risk of impurity generation during formulation process also gets elevated due the instable nature of Orlistat [Kothamasu et al, 2009].

CHAPTER 2

REVIEW OF LITERATURE

2.1 Solid Lipid Nanoparticles (SLNs)

The solid lipid nanoparticles were innovated in the year 1991 as an alternative approach for traditional colloidal carriers such as liposomes, polymeric microparticles, emulsions and polymeric nanoparticles [Ekambaram et al, 2012], Since then the drug delivery system has drawn so much attention of the researchers for the intravenous route of application as it can act as the perfect substitute of particulate colloidal drug carrier system [Gasco, 1993]

The solid lipid nanoparticle system comprises of the nanosized range spherical solid-lipid particles. The average size of the diameter of these nanoparticles ranges from 10 to 1000 nanometers. These particles are dispersed into water or any other aqueous surfactant solution [Ahlil et al, 1998]. The SLNs are made of the hydrophobic core of lipid which remains solid at room temperature and has the phospholipid monolayer coating over it. The hydrophobic solid core consists of the drug which is dispersed or dissolved into the solid matrix of the lipid and the phospholipid hydrophobic chains gets embedded into this solid lipid matrix. This system has the capacity to carry both hydrophilic as well as hydrophobic drugs and diagnostics in it [Shah et al, 2011]. The structure of SLN is shown in the figure 2.1.

Fig.2.1 Structure of Solid Lipid Nanoparticle

The solid lipid nanoparticles consist of the combined properties of fat emulsion, liposomes and polymeric nanoparticles. They possess several advantages such as non-toxic nature, better bioavailability, chemically stability from hydrolysis, biodegradability, coalescence, physical stability and efficient lipophilic drug carrier [Cavalli et al, 2002]. The main difference between the liposomes and the lipid emulsion is that the basic structure of lipid emulsion consists of a neutral hydrophobic oil core covered with a amphiphilic lipid

monolayer while the liposomes comprises of the amphiphilic phospholipid bilayer as outer covering and has aqueous chamber inside [Jain N.K., 1997].

The solid lipid nanoparticles can be administered through many routes as shown in Fig. 2.2 and their *in-vivo* activity depends on these routes of administrations.

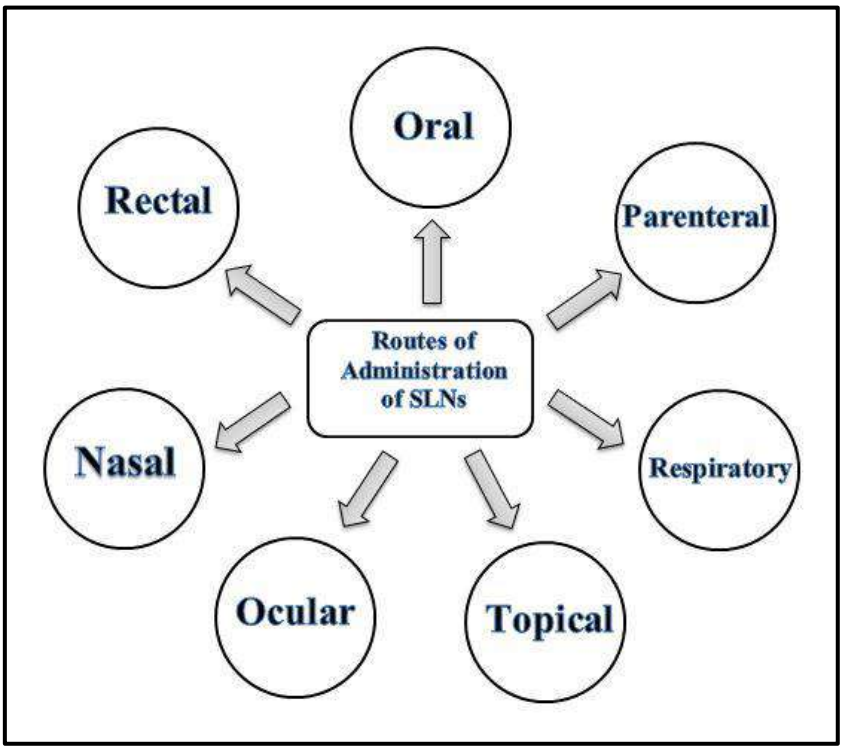


Fig. 2.2 Routes of administration of SLNs

2.1.1 Advantages of SLNs

- SLNs exhibits much better stability and gives better scope of upgradability as than that of the liposomes
- The lipid matrix of the SLNs consists of the physiological lipid which lowers the risk of either acute or chronic toxicity.
- The SLNs possesses long term and high physical as well as chemical stability.
- The manufacturing of solid lipid nanoparticles is comparatively easier than the preparation of bipolymeric nanoparticles.
- The solid lipid nanoparticles have good control over the release kinetics of the entrapped active component.
- SLNs are capable of improving the bioavailability of the incorporated bioactive compound.

- The solid lipid nanoparticles provide chemical stability to the labile encapsulated component.
- The solid lipid nanoparticles do not need any different raw materials than the emulsions. Therefore, they are easy and manageable to manufacture.
- The large-scale production of solid lipid nanoparticles is possible.
- The functional compound of solid lipid nanoparticle can be achieved at higher concentrations.
- The solid lipid nanoparticles are suitable to undergo lyophilisation, if needed. (Ramteke et al, 2012)

2.2 Methods of preparation of SLNs

There are several production methods through which we can achieve the solid lipid nanoparticles at large scale production [Olbrich et al, 2002]. Some of these approaches which are adopted in this work are:

- High pressure homogenisation
    - Hot high pressure homogenisation
    - Cold high pressure homogenisation
- Microemulsion
- Ultrasonication
- Solvent evaporation

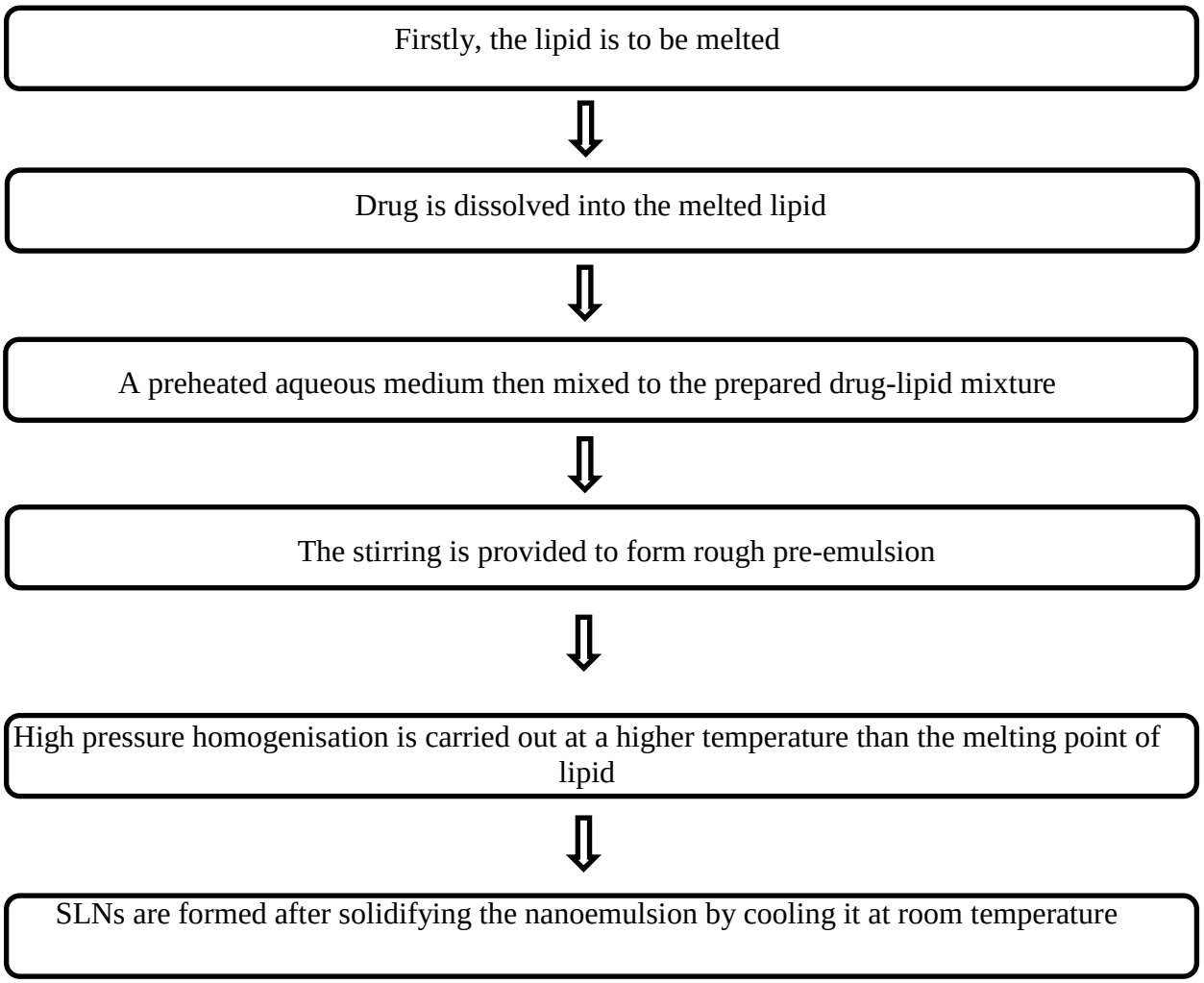
2.2.1 High Pressure Homogenisation

High Pressure Homogenisation (HPH) is an influencial and reliable technique for the preparation of solid lipid nanoparticles. In this approach, the high-pressure homogenizers thrust the liqiuid at a very high pressure of 100-2000 bar through a slender gap of few microns range size. This elevates the fluid to very high velocity of over 1000 Km/h on a very short distance. The very high shear stress and cavitation forces produced by these conditions leads to the disruption of particles to submicron range [Ekambaram et al, 2012].

At first, the HPH technique was adopted for the formulation of solid lipid nano-dispersions [Speiser et al, 1990; Domb et al, 1993]. However, the quality of the dispersion was often compromised due to the micro size range of the particles.

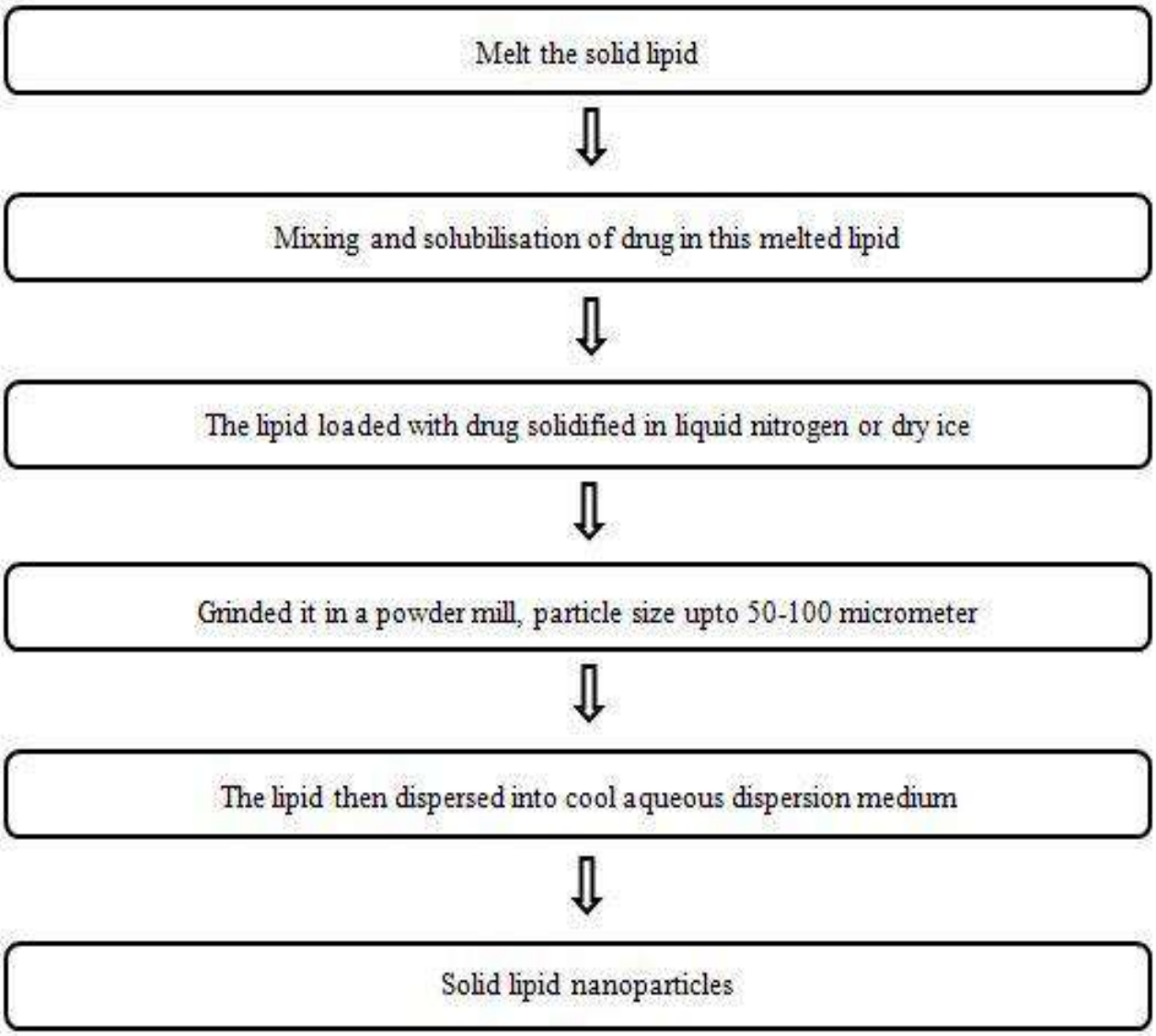
The HPH technique is carried out by two general approaches of hot high pressure homogenisation and cold high pressure homogenisation working on the same theory of mixing the drug into the bulk amount of melted solid lipid [Lander et al, 2000].

2.2.1.1 Hot High Pressure Homogenisation



The desired drug which is to be encapsulated into the nanostructured lipid carriers, was firstly dispersed or dissolved either into the melted lipid which was solid at the room temperature or into the mix of an oil (liquid lipid) and a melted solid lipid. Now, in the hot homogenization process, the hot lipid melt having the dissolved drug would be dispersed into a hot solution of surfactant with continuous and vigorous stirring. The temperature must be maintained 5-10 $^{\circ}$C higher than the melting point of the solid lipid or the whole lipid blend. Now this pre-emulsion should be treated under the high pressure homogenizer regulated to the same temperature as earlier and the cycles as three cycles adjusted at 500 bar or two cycles adjusted at 800 bars. The technique in mainly used in case of lipophilic and poorly soluble drugs [Gohla et al, 2001]. Since, the operating time and heat exposure time is short, some heat sensitive or thermolabile drugs can also be safely handled. But this techniques is not useful in case of hydrophilic drug incorporation into SLNs as the higher fraction of drug content in water at the time of homogenization will lead to low entrapment efficiency [Schwarz et al, 1994].

2.2.1.2 Cold High Pressure Homogenisation

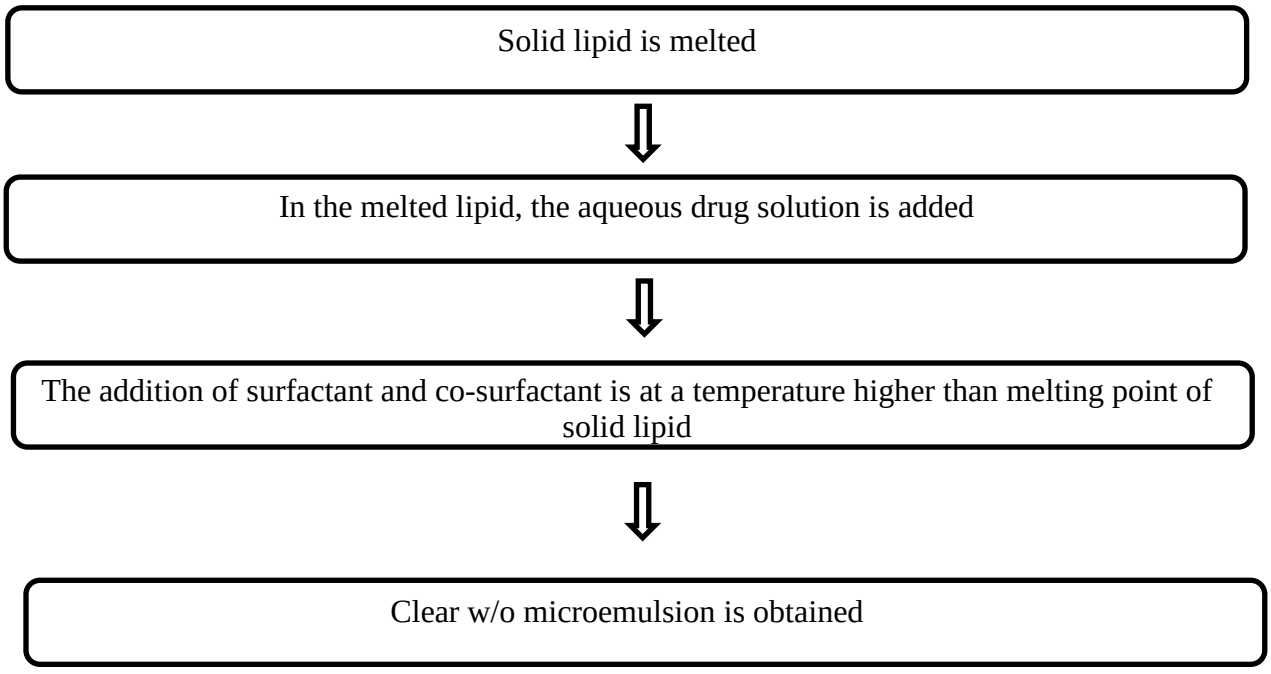


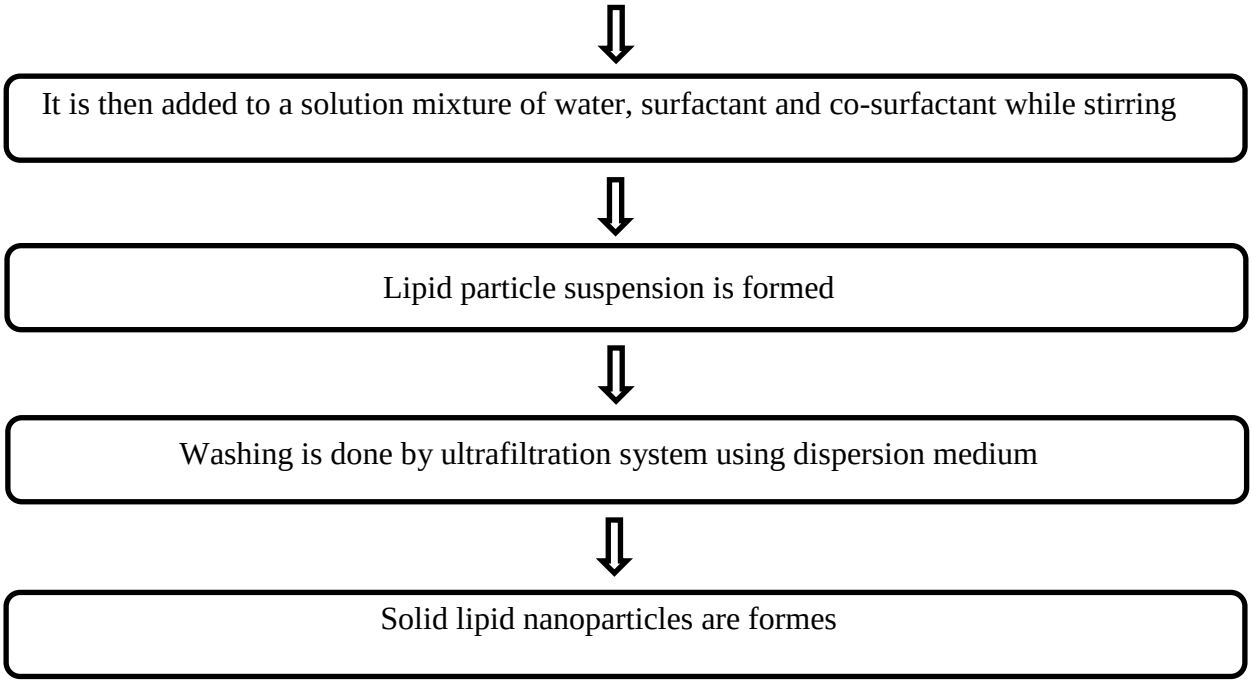
The initial step of cold high pressure homogenization is similar to that of the hot high pressure homogenization, that is, dispersion or solubilisation of drug into the pre-melted solid lipid or to the mix of a liquid lipid and a melted solid lipid. Then the prepared mixture is cooled promptly using dry ice or liquid nitrogen. The resulting solid product of SLN loaded with the drug was milled with the help of ball mill or mortar pestle up to the size of 50-100 micron and these milled microparticles then added into the cool emulsifier solution. The makes the pre-suspension. Followed by pre-suspension which is passed through the high-pressure homogenizer at or below room temperature where the cavitation process gets introduced strongly on the pre-suspension and breaks the microparticles further to the desired solid lipid nanoparticles. This technique reduces the lipid melting and thus lowers the risk of

drug loss of hydrophilic nature into the aqueous phase. A different approach can also be applied to ensure the minimum hydrophilic drug loss into the aqueous phase is by substituting the water with any other media for example, oil or PEG 600 in which the drug would be less soluble. The polydispersity index and particle size of product obtained from the cold HPH technique is more as that of the hot HPH technique. The cold HPH technique decreases the heat exposure time for the drug but does not avert it completely since the heat is applied for the melting of the solid lipid in the initial step as well as the high-pressure homogenization also generated heat during its vigorous cycles, that is, 10-20°C elevated temperature in each cycle. Mostly, 3-5 homogenization cycles at the pressure of 500-1500 bars are enough to produce desired solid lipid nanoparticles. Any increase in the pressure or the number of cycles in homogenization may lead to the larger particle size of the product caused by the high kinetic energy among the particles [Rabinarayan et al, 2010].

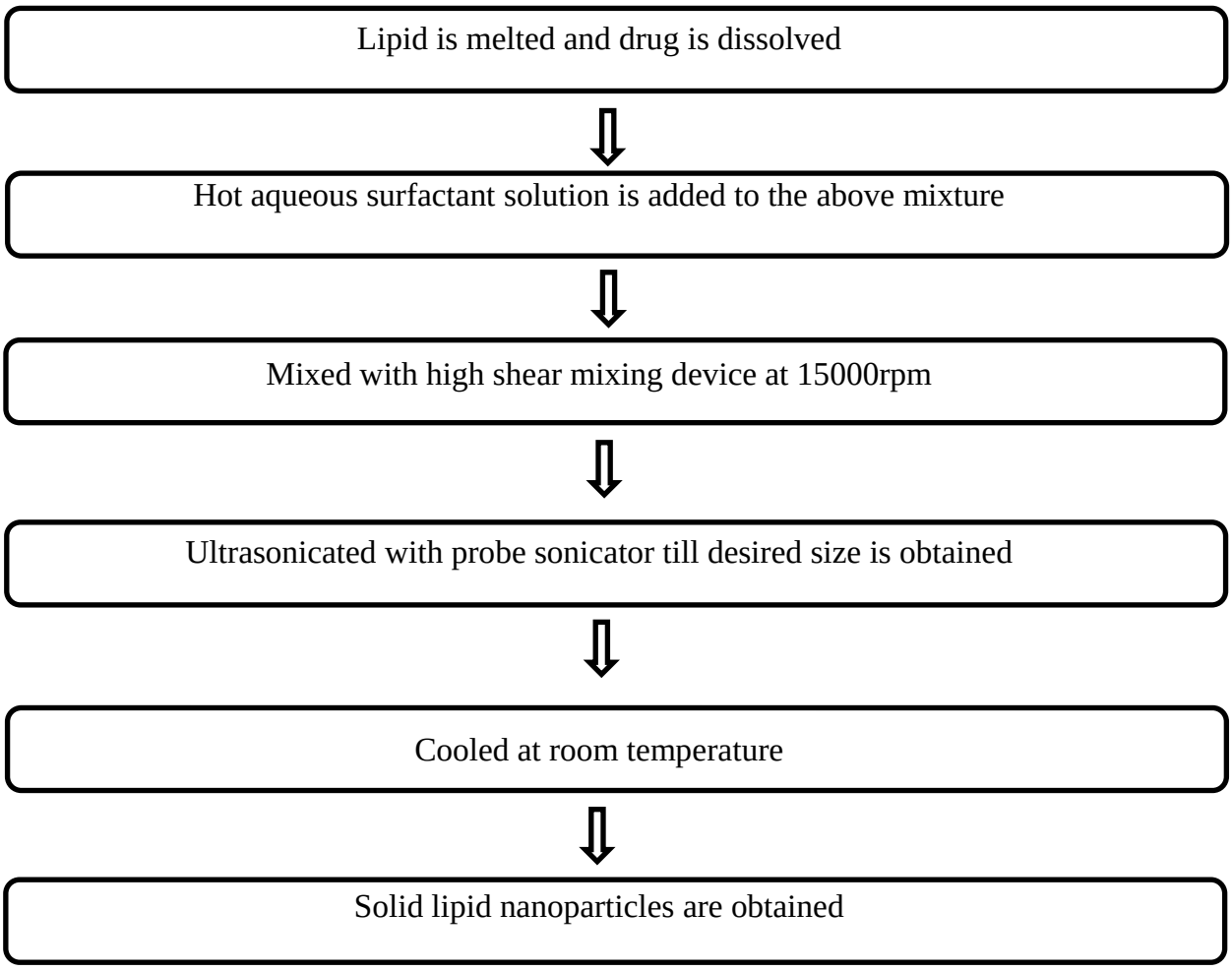
2.2.2 Microemulsion based SLNs

The principle of the technique is based on the dilution of microemulsions. The microemulsion is composed of two different phases, that is, one inner phase and one outer phase (o/w microemulsions). An optically transparent mixture was prepared containing a fatty acid with low melting point such as stearic acid, an emulsifier like polysorbate 20, a co-emulsifier for example butanol and water. This mixture is then stirred continuously at 65-70°C temperature. Then this hot mixture is mixed into cold water having temperature 2-3°C during stirring. The rapid change in the temperature helps in fast lipid crystallisation and prevents the risk of accumulation. The presence of lipid content in the microemulsion is comparatively less as that of the HPH formulations because of the dilution step [Jain, 1997]



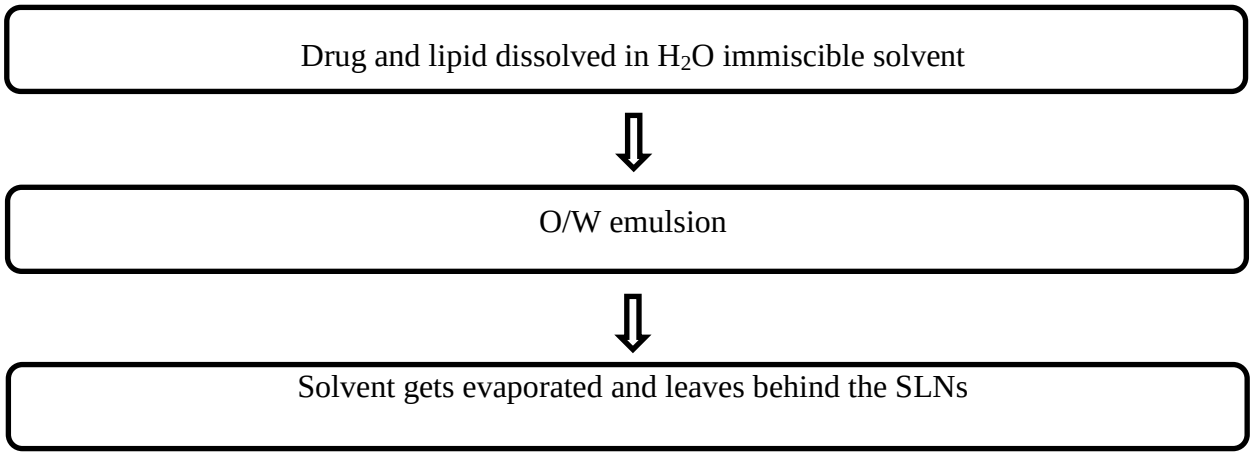


2.2.3 Ultrasonication (Speicer, 1990; Eldem et al, 1991)



2.2.4 Solvent Evaporation

The hydrophobic drug and the lipophilic material are dissolved in a non-polar solvent like cyclohexane, chloroform, toluene etc. and then emulisified in an aqueous phase during high speed homogenization. For the enhanced efficiency of the emulsification, the rough or coarse emulsion is passed through the micro fluidizer immediately. The organic solvent gets evaporated during stirring at reduced pressure and room temperature leads to lipid precipitates of SLNs (Siekmann et al, 1996).



2.3 Work done on Solid Lipid Nanoparticles

Yang et al, 1999 developed the solid lipid nanoparticles loaded with Camptothecin for the oral route of administration and investigated for changes in the action of SLN formulation of Camptothecin over the distribution of the drug into the body. These Camptothecin-SLNs were prepared using the technique of high pressure homogenisation and the polymer Poloxamer 188 was used for the coating of SLNs. The characterisation of the prepared SLNs was done by electrophoretic mobility measurement and electron microscopy. Also, the *in-vitro* release studies were carried out in several different pH media. The concentration of drug contents was determined in the organs after the oral administration of the prepared formulation of Camptothecin SLNs and a Camptothecin control solution with the help of reversed-phase high performance liquid chromatography having a fluorescence detector. As a result of the studies it was found that the Camptothecin-SLNs particles had the average diameter of 196.8 nm along with the Zeta Potential -69.3 mV while the entrapment efficiency was the drug was upto 99.6% and the *in-vitro* drug release was sustained about a week. In the organ testings the mean residence time and area under curve of the formulated Camptothecin-SLNs increased remarkably as that of the drug control solution. The highest increased AUC was noted in the brain. Therefore, the study concluded that the solid lipid nanoparticles of

Camptothecin or similar lipophilic drugs are capable of providing reliable targeting and sustained release system when delivered orally.

Venkateswarlu et al, 2004 formulated and evaluated Clozapine solid lipid nanoparticles for the purpose of improvement in the poor oral bioavailability of Clozapine. The SLNs dispersion was prepared by performing hot homogenisation of lipid and aqueous phase and then the ultrasonication technique was carried out at the temperature higher than the melting point temperature of the lipid. These solid lipid nanoparticles were formulated with three different triglycerides namely, trimyristin, tripalmitin and tristearin along with other ingredients like Poloxamer 188, soylecithin 95% and stearylamine. The characterisation of the prepared formulation carried out by determining particle size and zeta potential using photon correlation spectroscopy (PCS) from Malvern Zetasizer and the drug was characterised by DSC and powder X-Ray diffraction. The *in-vitro* drug release studies were performed on Franz diffusion cell in 0.1N HCl, phosphate buffer pH 7.4 and double distilled water. The desired SLN formulation was obtained in a stable state with average size range of 60-380 nm and its zeta potential was found to be -23 to +33mV. The resulting entrapment efficiency of the drug enhanced to 90% and the DSC and PXRD reported the drug to be in amorphous form providing improved solubility and bioavailability of the drug. Weibull and Higuchi equations were found to be followed as the release pattern by the drug.

Casadei et al, 2006 designed and evaluated the solid lipid nanoparticles of ibuprofen incorporated into dextran hydrogels. The formulation was prepared taking Preciol ATO 5 acting as the lipid phase along with or without ibuprofen, undergoing hot high pressure homogenisation technique. The prepared formulation was then characterised through the measurement of particle size and zeta potential. Then the freeze-dried loaded SLN samples presented melting point shift of lipid phase higher than the melting point of empty SLN. After that, the dextan methacrylate was added into the aqueous phase and eventually obtained the loaded or unloaded SLN incorporated into the dextran hydrogel. From the freeze-dried samples the dissolution studies of the drug ibuprofen were performed and the release profiles of Ibuprofen in SLN, in dextran methacrylate and SLN-dextran methacrylate hydrogel were compared and it was found that the last system was the most suitable modified delivery system for oral formulations.

Sarmento et al, 2007 produced and characterized cetyl-palmitate based solid lipid nanoparticles incorporating insulin to investigate the oral administration capability of these colloidal carriers. The preparation of solid lipid nanoparticles was carried out using an

improved solvent emulsification evaporation method on the grounds of w/o/w double emulsion. The characterization was done and the average particle size of insulin-loaded and unloaded SLN was found to be 350 nm, their zeta potential was determined negatively charged and the association efficiency of insulin was more than 43%. When the insulin-loaded SLN was administered orally into the diabetic rats, a significant hypoglycaemic effect could be noticed within 24 hours of the administration. Therefore, this study concludes that SLNs encourages the oral absorption of insulin.

Khare et al, 2016 prepared and assessed the solid lipid nanoparticles of Voriconazole. The marketed products of Voriconazole are available for intravenous and oral route of administration. Voriconazole is a broad spectrum second generation antifungal drug and it also has various adverse effects in systemic administration such as hepatic and visual abnormalities. Therefore, in this study an attempt was made on the ocular delivery of Voriconazole loaded in solid lipid nanoparticles and its results were examined. The SLNs were prepared taking stearic acid for the lipid phase, stabilizer tween 80 and Carbopol 934 as the controlled release polymer, it also helps in improving the precorneal residence time of drug in eye. The process of SLN preparation was carried out by applying two different methods, one is ultrasonication technique and the other one is microemulsion method. The particle size in the resulting two preparations was found to be larger in the microemulsion formulation as compared to that of the ultrasonication technique product. The characterisation of all formulation gave the outcome that the polydispersity index of both the formulations were found to less than 0.3 and the PXRD and DSC of the drug confirmed the enhanced amorphous nature of the drug. The SLN formulation prepared by ultrasonication technique gave 12 hours of sustained release in the *in-vitro* drug release studies. Thus, it was concluded from this study that the ultrasonication method was more suitable and caused no significant harm on the corneal hydration level as compared to the microemulsion technique for SLNs.

Omwoyo et al, 2014 designed, characterized and optimized the Primaquine loaded solid lipid nanopraticles. Primaquine is the most common and extensively used antimalarial agent and has the potential to resist the recurrence of malaria but it is limited to lower doses as its higher doses results into severe tissue toxicity, gastrointestinal and hematological side effects. Thus, the nano delivery system was took up for the purpose of estimated improvement in bioavailability, therapeutic efficacy, significant dose reduction and therefore, reduced toxicity of Primaquine. The solid lipid nanoparticles loaded with Primaquine were prepared using the amended solvent emulsification evaporation method based on w/o/w double emulsion. The characterisation of the formulation was performed by determining various parameters. The

mean particle size was 236 nm, the zeta potential changed drastically from -6.54 mV to +23.0 mV when binded with positively charged chitosan acting as a surface modifier. The drug loading was estimated to be 14% and the drug encapsulation efficiency was found to be 75%. A steady release of up to 72 hours was observed during the *in-vitro* drug release studies. DSC showed the physical stability of the prepared formulation due to the vanished decomposition exotherms and the FT-IR confirmed the chemical stability by confirming no interactions between the drug and other components of the formulations. It was found in the test performed on the *Plasmodium berghei* infected Swiss albino rats that the prepared nanoformulation of Primaquine was 20% more efficient than the routine doses of Primaquine proving this solid lipid nanoparticle formulation of Primaquine to be the effective and better delivery system for the antimalarial drugs.

Khalil et al, 2014 prepared and assessed the Solid lipid nanoparticles loaded with Nystatin antifungal drug for the topical application. The solid lipid nanoparticles have achieved a revolutionary lead in topical administration of the drug by facilitating better skin penetration. In this work, the SLNs were prepared by carrying out the hot high pressure homogenisation and ultrasonication techniques. After the characterisation of the prepared formulation the particle size was observed to be in the range of 83.26 to 955.04 nm, the entrapment efficiencies were falling in the range of 19.73 to 72.46% while the zeta potential of the prepared formulation was found to ranging between -18.9 to -38.8 mV. The stability studies were carried out for 6 months and these studies depicted the stability of Nystatin and confirmed its efficiency in SLN formulation for topical use. In the microbiological test conducted on the male rats which were infected with Candidiasis albicans by examining any histopathological changes occurring on the skin of the rats and also by counting the colonies, it was determined that fewest number of colony forming unit/ml (cfu/ml) was observed in the prepared Nystatin solid lipid nanoparticle formulation as compared to that of the other conventional marketed products of Nystatin. Thus, it can be concluded from this work that the Nystatin loaded SLNs shows better skin targeting and enhanced therapeutic efficacy in fungal infections.

2.4 Orlistat

2.4.1 Therapeutic use

Orlistat is the saturated derivative of the irreversible inhibitor of pancreatic lipase Lipstatin, which is isolated from the bacterium *Streptomyces toxytricini* [Barbier et al, 1987]**.**

Orlistat is an anti-obesity drug which prevents the absorption of fat in the body and thus reduces the intake of calories. It has been reported that if it is used in combination with the low-calorie diet and appropriate physical exercises, many Kgs more weight loss would take place as compared to the weight reduction would occur without Orlistat over the time span of one year [Padwal et al, 2004]. Other than weight loss, Orlistat has the potential to reduce blood pressure as well as the commencement of Type 2 Diabetes Mellitus either with or without its weight-reducing properties [Torgerson et al, 2004]. It is capable of reducing the Type 2 diabetes risk as it impairs the glucose tolerance in the body and lowers the LDL-C levels to relatively greater extent than the weight loss alone. Orlistat is considered as a useful drug in the weight management therapy of patients who also have the risk of cardiovascular disorders along with obesity due to the fact that when it is given in the combination with statins, ezetimibe and fenofibrate it results into increased degradation of LDL-C levels as compared to their reduced levels in monotherapy of these drugs [Baigent et al, 2005]. Furthermore, the combination with ezetimibe or fenofibrate leads to the decrease in concentration of the atherogenic, dense, small LDL-C, it decreases the total plasma lipoprotein- associated phospholipase A2 activity and improves various cardiovascular risk factors than the therapy of individual drug [Filippatos et al, 2009].

2.4.2 Mechanism of action

The dietary fats gets digested by the gastric and pancreatic lipase enzymes present in the GI, this digested fat gets absorbed into the body and gets accumulated which results into obesity and also causes the risk of high blood pressure and various heart diseases. Orlistat, the lipstatin semisynthetic derivative is an efficacious and selective inhibitor of these gastric and pancreatic lipase enzymes with causing negligible or absolutely no harmful effect over other enzymes such as amylase, phospholipases, chymotrypsin and trypsin and keeps its activity and effects exerted not beyond the GI tract. Orlistat is capable of reducing the dietary fat absorption up to 30% [Hauptman et al, 1992]. The mechanism of action is depicted in a diagram in Fig 2.3.

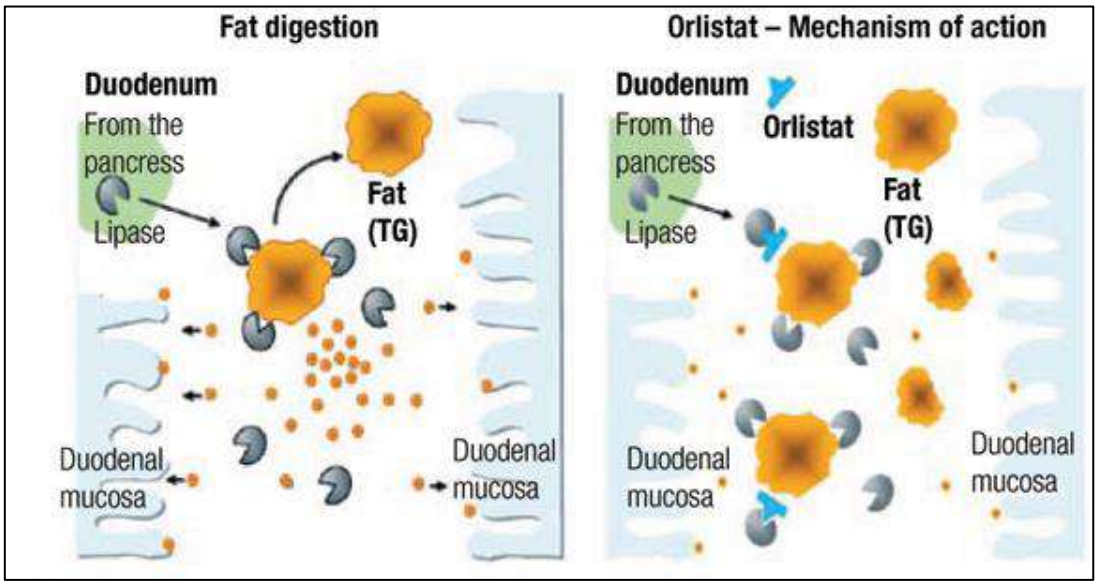


Fig. 2.3 Mechanism of action of Orlistat

On the active sites of pancreatic lipases, Orlistat makes a covalent bond with the serine residues. When Orlistat is taken along with the fatty diet, it partially constrains the hydrolysis process of triglycerides leads to the reduction of the following absorption of free fatty acids and monoacylglycerides. The pharmacological effect of Orlistat is dose-dependent and it neither interfere into other GI physiological activities like acidity, gastric emptying, bile composition, lithogenecity and gallbladder motility nor into the systemic mineral and electrolyte balance. Also, Orlistat has no tendency to affect the pharmacokinetics and absorption of drugs having small therapeutic index such as phenytoin, digoxin and the commonly used compounds by obese people, that is, pravastatin, delayed release nifedipine, oral contraceptives [Guerciolini R, 1997].

2.5 Obesity

According to the World Health Organisation, overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health.

To classify the overweight and obesity, BMI (Body Mass Index) is determined which is a weight-for-height scale. To calculate the BMI of any person, his/her weight (in Kg) has to be divided by the square of his/her height (in meters) i.e. kg/m^2.

Following are the parameters to determine obesity:

In case of adults,

- Overweight – if the BMI $\geq$ 25; and

- Obese – if the BMI ≥ 30

The BMI is the most efficient multitude-level scale of obesity and overweight since it is determined equally for all the ages in adults and for both the sexes. Although, it might not be corresponding up to the same level of fatness in different people.

For kids, age should be considered as an important factor while determining obesity and overweight.

In case of children under the age of 5 years,

- Overweight – if the BMI > 2 standard deviations above WHO Child Growth Standard median; and
- Obese – if the BMI > 3 standard deviations above WHO Child Growth Standard median.

In case of children between 5-19 years of age,

- Overweight – if the BMI > 1 standard deviation above the WHO Growth Reference median; and
- Obese – if the BMI > 2 standard deviations above the WHO Growth Reference median [WHO, feb 2016]

2.5.1 Causes of obesity

The common causes of obesity are:

- ✓ Excessive food intake
- ✓ Less or no physical activity
- ✓ Genetics: The genetic factor is one of the common obesity causes. Not much can be done regarding that other than regular exercise and controlled diet. The family history of hypothyroidism, diabetes, hypertension etc. may also result into obesity.
- ✓ High carbohydrate and fatty diet
- ✓ Medications: Some medications play significant role in weight gain such as diabetic treatments, steroid hormones, psychotropic medications, antihypertensive drugs, contraceptives, protease inhibitors and antihistamines. The drug-induced obesity comes with further risks of enrooting hyperlipidemia, hypertension, type II diabetes along with poor compliance to medications [Aronne et al, 2003].
- ✓ Mental illness: Psychological factors like stress, depression, anxiety may results into eating disorders also known as stress eating or comfort eating which eventually displayed as obesity.
- ✓ Lifestyle: The modernized lifestyle with more mechanized work and ease of technology results in lesser physical activity. The availability of processed food in order to make

things quick and easy, the consumptions of saturated fats and synthetic food has increased tremendously leading to deposition of body fat. Lack of sleep is also one of the lifestyle factors which affects the biological cycle of the body which when gets disturbed, in order to compensate the stress body tends to produce more and more body fat.

- ✓ Diseases: Diseases like hypothyroidism, polycystic ovary syndrome, Cushing's syndrome, insulin resistance etc. may also result in obesity [Flegal et al, 2010].

Obesity itself is a serious issue but it also eventually causes the risk of developing various other health problems as shown in Fig. 2.4.

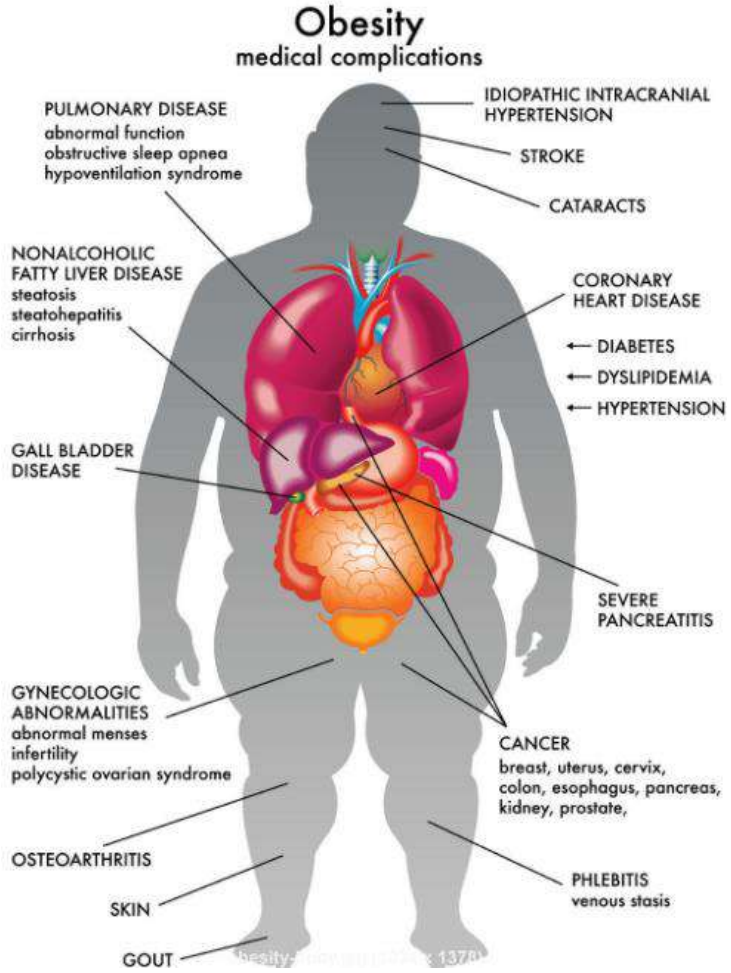


Fig 2.4 Medical complications due to obesity

2.5.2 Obesity management

The obesity and overweight issues are greatly preventable as well as reversible. Therefore, the obesity management is possible and effective. It primarily includes physical exercise and proper healthy diet [Lau et al, 2007]. The diet programs alone can work for a short period of time and may result into weight loss but in the long run, maintaining it can be difficult. Hence, the combination of low calorie diet and regular exercise must carry out for the permanent weight loss effect [Shick et el, 1998].

In order to reduce weight, one can:

- ✓ Limit the intake of processed food and avoid having sugar and fats.
- ✓ Switch to vegetables and fruits instead. Consume legumes, nuts, whole grains, fibres; and
- ✓ Engage one's self in regular exercise of atleast 60 minutes for kids 150 minutes for an adult per week.
- ✓ Encourage yourself to opt for a scheduled day and get 7-8 hours of sleep every day to provide the much needed rest to the body.
- ✓ Try meditation to avoid stress and hypertension, especially those who suffers from depression and anxiety.

2.6 Work done on Orlistat

Jain et al, 2006 prepared and evaluated the porous carrier based floating Orlistat microspheres for gastric delivery. The solvent evaporation method was chosen for the preparation of floating microspheres and their gastro-retentive and controlled-release properties were evaluated. The optimization was done by studying the particle morphology, *in-vitro* drug release, micromeritic properties, percentage drug entrapment and *in-vitro* floating behavior of the microspheres. The transit time of floating microspheres was monitored on albino rabbits by the gamma scintigraphy and the pharmacokinetic parameters of Orlistat were determined from the blood samples of albino rabbits in which the formulation was administered orally. The formulation containing 200mg calcium silicate gave best-floating property and the release pattern of Orlistat floating microspheres followed Higuchi matrix model and Peppas-Korsmeyer model in all formulations. The calcium silicate based floating microspheres exhibited increased gastric retention time of up to 6 hours along with improved elimination half-life.

Dolenc et al, 2010 designed and evaluated nanosuspension of Orlistat, intended to enhance its *in-vitro* dissolution rate and examining any improvement in the lipase-inhibiting properties of the anti-obesity agent. This work was done to consider the issues that may occur in the formulation of controlled particle size of the nanosuspension due to the unmanageable waxy nature, poor chemical stability and low melting point of Orlistat. The process was carried out using high-pressure homogenization and melt emulsification techniques. Lactose was used as a filler and dissolved into the nanosuspension in order to prepare a dry formulation followed by the spray drying to get the final product. For the characterization of Orlistat nanosuspension, scanning electron microscopy, laser diffraction and atomic force microscopy were performed and the *in-vitro* efficacy of the formulation was characterized by the carrying out its *in-vitro* dissolution and lipase inhibition studies. The techniques used for the

preparation of nanosuspension of Orlistat results in significant increase in its *in-vitro* dissolution rate as compared to that of pure drug, the physical mixture or any marketed formulations. This formulation also demonstrates the remarkable improvement in the pharmacological effect of Orlistat which in turn reduces the dose and thus lessens the side effects caused due high doses.

Singh A.V., 2011 formulated and evaluated the solid dispersion of Orlistat using poloxamer 188 as a hydrophilic carrier by picking the kneading method intending to enhance the solubility and dissolution properties of Orlistat. The physicochemical characterization was carried out along with the *in-vitro* dissolution studies of the solid dispersion of Orlistat. The change in the crystalline arrangement was observed using FT-IR and DSC and resulted in its conversion to its amorphous form which is rather easy to get solubilized and the *in-vitro* dissolution studies of the physical mixture as well as solid dispersion were found to be much more improved as compared to the pure active drug. It was concluded that the Orlistat solid dispersion consisting 1:5 ratio of drug and hydrophilic carrier would give solubility and dissolution which in turn also gives improved bioavailability.

Samyukta et al, 2011 preparation and *in-vitro* evaluation of niosomes of Orlistat for the purpose of increasing the poor and variable bioavailability of this anti-obesity agent. The process of preparation of non-ionic surfactant vesicles was carried out using reverse phase evaporation technique i.e., the slurry method. The β-cyclodextrin and Span 60 were mixed and prepared a slurry of them followed by drying in rotary flash evaporator producing a free flowing powder which when added into a buffer for rehydration. The selected component of the lipid mixture was cholesterol and span 60 and β-cyclodextrin carries were taken in various molar ratios. The characterization and evaluation of the niosomal formulation were carried out by studying its entrapment efficiency, particle size, drug release kinetics, surface morphology, conductivity, stability studies, viscosity, pH, density and sedimentation rate studies. The release kinetic was studied by Hixson model and the mechanism behind the release was found to be diffusion. The FT-IR reports no interaction between the drug and the excipients and the SEM images revealed the mean niosome size was 100 nm with a smooth surface. The prepared niosomal formulation exhibited stability at room temperature for 90 days. Thus, the niosomal drug delivery system proved to be a suitable for Orlistat, providing better bioavailability and stability.

Sangwai et al, 2012 innovated nanoemulsion of Orlistat and transformed it into multi-unit pellet system (MUPS) seeking improved dissolution and pancreatic lipase inhibition. This delivery system draws attention to the enhancement of the oral delivery of this poorly water soluble anti-obesity drug Orlistat. The nanoemulsion was prepared by high-pressure homogenization technique using Capryol PGMC as oil phase and Cremophor RH40 as the emulsifier. The globule size distribution, physical stability and polydispersity index were evaluated. Then this optimized nanoemulsion was transformed into MUPS with extrusion spheronization technique. The characterization of the optimized formulation was done on both nanoemulsion as well as MUPS stages. The dynamic light scattering (DLS) and tracking studies of nanoparticles shows the unimodal size distribution and polydispersity index. The uniform and spherical nanosized oil droplets were confirmed with confocal laser scanning microscopy of the nanoemulsion. In MUPS characterization, PXRD and DSC studies reveal the conversion of nanoemulsified Orlistat in amorphous form. The *in-vitro* dissolution rate of the prepared nanoemulsified MUPS of Orlistat was reported to be remarkably improved as that of the pure drug as well as the marketed product and the pancreatic lipase inhibition activity of Orlistat were when compared to that of the pure drug and marketed formulation, was found to be 13.57 and 2.41 folds higher. Respectively.

Desai et al, 2012 designed, developed and optimized self-microemulsifying drug delivery system of Orlistat. The solubility studies were performed on oils, surfactants and co-surfactants in order to select the components of the self-emulsifying drug delivery system (SMEDDS) of Orlistat. After the selection of components, different formulations were estimated by plotting pseudoternary phase diagram and determining the microemulsification area. The characterization of the prepared formulation was carried out by determining their zeta potential, dispersibility test, particle size and thermodynamic stability studies. The thermodynamically stable formulations were then taken for *in-vitro* dissolution studies and the results were compared to that of the pure drug and suspension formulations. The SMEDDS of Orlistat gave improved dissolution rate and thermodynamic stability and thus it can be concluded that the hydrophobic drugs such as Orlistat can be efficiently delivered through self-microemulsifying drug delivery system.

Gade et al, 2016 formulated, evaluated and optimized the self-emulsifying tablets of Orlistat for the enhancement of its *in-vitro* drug release along with the *in-vivo* anti-obesity activity studied on Wistar rats. First of all, the solubility of Orlistat was determined in various natural oils, surfactants and co-surfactants. The selected components of self-emulsifying drug

delivery system (SEDDS) of Orlistat were turned out to be Castor oil, Tween 80 and Capryol PGMC. The globule size and emulsification time were evaluated in liquid SEDDS. The optimization was done using 3^2 full factorial design. After the optimization of the formulation, the one with minimum globule size undergone through the freeze-drying process and the resulting powdered product was compressed into SEDDS tablets. The FT-IR studies indicated no chemical interaction between Orlistat and other excipients while the Differential Scanning Calorimetry and X-ray diffraction reveal the conversion of the crystalline form of Orlistat into the amorphous form. The prepared self-emulsifying tablets showed the higher *in-vitro* release of Orlistat and gave improved *in-vivo* weight-reducing activity on Wistar rats.

2.7 Optimization of formulation (DoE) by using Central composite design

The optimization of formulation or process by using Design of expert involves the overall interpretation of results in the form of graphs as well as mathematical equations with respect to the variables entered as input [Armstrong, 2006; Lewis et al., 1998; Singh et al., 2011]. The independent variables are the factors which are under the control of the formulator and are not dependent on any other factor whereas, the dependent variables are those variables which are obtained as the responses which are a measure of outcome from the experiment. The independent variables or factors are generally symbolized as X variables and the responses as Y variables. The experimental design is selected according to the desirability to obtain the efficient and precise results. According to the selected statistical approach the relationship between the independent and the dependent variables is established. The depiction of the mathematical relationship in the form of 3-D graph is known as response surface plot. The response surface is helpful in understanding the relationship between the independent variables and the responses. Other than the 3-D graph, contour plots play a significant role in determination of geometric response and relationship of independent variable and dependent variable by keeping the magnitude constant. The response surface design generates an area of investigation in which the main effects, interactions as well as quadratic effects can be investigated on the basis of the shape provided with the higher and lower limits. The use of response surface design helps to find the improved optimized formulation or process against the effect of some noncontrollable influences. The RSM predicts the most suitable result out of the input data which can further be validated experimentally. The error between the predicted and experimental results can be determined by the factor called prediction variance. Thus, the suitability of the design can also be checked by the value of prediction variance which should fall within the limits for acceptance of the experimental method for analysis.

EXPERIMENTAL DESIGNS

There are different types of designs which are available for the optimization process. Depending upon the need, the most suitable optimization design is selected and evaluations are carried. Following are the experimental designs:

a. Factorial designs

b. Fractional factorial designs

c. Plackett- Burman designs

d. Star design

e. Central composite designs

f. Box-Behnken designs

g. Center of gravity designs

h. Equiradial designs

i. Mixture designs

j. Taguchi designs

k. Optimal designs

l. Rechtschaffner designs

m. Cotter designs

2.7.1 Selection of Central composite design for optimization

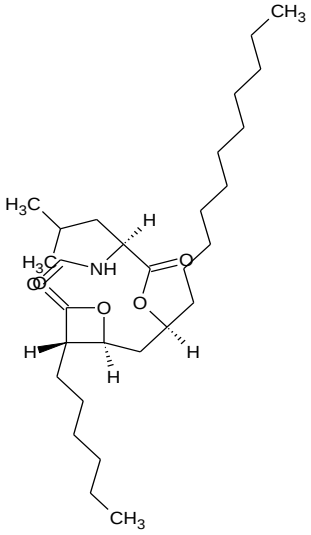
Central composite design (CCD) is the most frequently used design for the non linear second order models. The "composite design" contains an imbedded (2k) factorial design (FD) or (2 k-r*)* fractional factorial design (FFD), augmented with a group of star points *(2k)* and a "central" point. The star points help in the estimation of curvature and to show the high and low limits. A face centered cube design (FCCD) results when both factorial and star points in a CCD possess the same positive and negative distance from the center. Circumscribed central composite designs are the original form of the central composite design. The star points are at some distance from the center, based on the properties desired for the design and the number of factors in the design. These designs have circular, spherical, or hyperspherical symmetry, and require 5 levels for each factor.

The composite designs normally involve the investigation of X at five levels—i.e., one central point (0 level), two factorial points (+1 levels) and two axial star points (±1 levels). However, in case of FCCD, the number of levels is kept at three for each factor. The advantage of using the central composite design is that it extrapolates the limits in all the

directions for finding the optimized results. The chances of finding the optimized results within the limits and with the extrapolated limits get increased. Computer softwares have been used at every step of optimization from selection of design to interpretation of results and validation [Lewis et al., 1998; Singh et al., 2011]. Use of computer software make the optimization easier and faster. Various software like Design expert, JMP, SOLVER, COMPACT, SPSS etc are used for the optimization process of various process and formulation parameters.

2.8 Drug profile of Orlistat

Table 2.1

Parameter	Description
Drug Name	Orlistat
Chemical structure	
Molecular weight	495.735g
Chemical formula	$C_{29}H_{53}NO_5$
IUPAC Name	(S)-((S)-1-((2S,3S)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl)2-formamido-4-methylpentanoate
Appearance	White Crystals
Pharmacology	Obesity management, inducing weight loss and its maintenance when used in conjointment with reduced-calorie diet.
Pharmacodynamics	Orlistat inhibits the pancreatic lipase enzyme which helps in breaking down the fats in the intestine. The inhibition of this enzyme leads to the excretion of fats without getting digested and no absorption of fats would occur in the body.
Oral absorption	Negligible
Protein binding	>99%
Metabolism	In the GI tract
Route of elimination	Major route of elimination is Faecal but it can also be eliminated through biliary excretion.
Mean plasma half life	1-2 hours
Dosage forms	Capsule, hard- 60mg, 120mg Chewable tablets- 27mg
Melting range	41-43°C

CHAPTER 3

RESEARCH ENVISAGED AND PLAN OF WORK

3.1 Rationale

Orlistat is a poorly water-soluble drug, it belongs to the category of BCS Class II drug which means the drug has low solubility and high permeability. It is an anti-obesic agent and is used in the management and treatment of obesity along with the healthy diet and physical activities [Pommier A et al, 1995]. The drug shows an effective pharmacological effect. Due to the poor water solubility, Orlistat offers limitations related to oral absorption and bioavalability, it needs a suitable delivery system which can help to overcome these complications and enhance the bioavalability as well as reduce the dose to decrease the risk of side effects.

Orlistat inhibits the pancreatic lipase enzyme which breaksdown the fats in the stomach which further gets accumulated in the body, these enzymes are mainly present in the pancreas and the stomach. Furthermore, the metabolism of Orlistat takes place in the gastrointestinal tract. Therefore, the drug has to be administered orally to get the desired pharmacological action.

Various oral preparations which have been reported to overcome these problems includes: floating tablets of Orlistat which can enhance the contact time of drug in stomach but does nothing to elevate the dissolution of drug in the body. The noisomal preparation which gives very slow release of drug in the bodyand can show the effect of drug for longer duration. The drug has to be taken with every significant meal i.e. thrice a day and it has to work accordingly [Samyukta R.B et al, 2011].

Thus, from the governed studies, it can be assessed that modification in drug delivery carrier can serve the desirable goal and the present study an attempt has been made by designing of SLNs for Orlistat. Solid lipid nanoparticles are the nano range lipid particles developed in an aqueous phase encapsulating the drug. The nano range enhances the surface area and the lipid particles dispersed in aqueous phase will act as a bridge in dissolving the drug into the body quickly and will help in achieving the desired oral absorption and bioavailability.

3.2 Aim and objectives

3.2.1 Aim of work

The aim of the presented work was “Formulation development and evaluation of solid lipid nanoparticles loaded with Orlistat for oral drug delivery”.

3.2.2 Objectives

- To encapsulate Orlistat into solid lipid naoparticles and develop an oral dosage form with high drug payload for improved efficiency.
- To enhance the oral absorption and bioavailability of drug in the body and reduce the dose to minimise the side effects.

3.3 Comprehensive plan of work

- Selection of excipients like lipid, surfactant, co-surfactant and stabilizer.
- Preformulation studies: compatibility study, solubility analysis, partition coefficient, prescreening studies for development of formulation.
- Development of solid lipid nanoparticles containing drug by optimizing various chemical and physical variables by optimization technique.
- Physical and chemical characterization of SLNs with respect to entrapment efficiency, particle size analysis, transmission electron microscopy, *in vitro* drug release studies.
- Incorporation of solid lipid nanoparticles into capsules and characterization including stability studies, *in-vitro* dissolution studies, comparative studies with the marketed formulation.

CHAPTER 4

MATERIALS AND METHODS

4.1 List of materials and equipment used in the study

Table 4.1

List of materials used in study

S.No.	Chemical/Material	Batch Number	Source/Manufacturer
1	Orlistat	ORL160917	Bills Biotech Pvt. Ltd., Vadodara, Gujarat
2	Glyceryl Monostearate	14277	B.B. Chemical Industry, Amritsar, Punjab
3	Tween 80	18304	B.B. Chemical Industry, Amritsar, Punjab
4	DCM	00094	Loba Chemicals Pvt. Ltd, Mumbai, Maharashtra
5	Soy Lecithin	23876	Himedia Laboratories, Mumbai, Maharashtra
6	Iodine	91030211J13	Finar Limited, Ahmedabad, Gujarat
7	Chloroform	0007502500	Loba Chemicals Pvt. Ltd, Mumbai, Maharashtra
8	Polyvinyl Alcohol	MKM250709	Qualikems Pvt. Ltd, Vadodara, Gujarat
9	Lactose Monohydrate	04330	Loba Chemicals Pvt. Ltd, Mumbai, Maharashtra
10	Talc	17957	B.B. Chemical Industry, Amritsar, Punjab
11	Hard gelatine capsule shells	NA	Lovely Professional University, Chemical store

Table 4.2

List of equipment/software used in the study

S.No.	Equipment	Model Number	Manufacturer
1	Hot air oven	Q-5247	Navyug, Mumbai, Maharashtra
2	Heated/Magnetic stirrer	2 MLH	Remi Pvt. Ltd, Mumbai, Maharashtra
3	Electronic weighing balance	CY360	Shimadzu Co. Pvt. Ltd, Japan
4	FTIR spectrometer	Spectrum 400	Shimadzu Co. Pvt. Ltd, Japan
5	Melting point apparatus	NA	Sanjay Biological Museum, Amritsar, Punjab
6	Bath sonicator	NA	Raj Analytical Services, India
7	Homogenizer	2500 rpm	Remi Elektrotachnik Limited
8	UV Spectrophotometer	2M9F365001	Shimadzu Co. Pvt. Ltd, Japan
9	Trinocular microscope	10390	Getner instruments Pvt. Ltd. in collaboration with Kyowa, Japan
10	Water bath shaker	NSW 133	Narang Scientific Works, Mumbai, Maharashtra
11	Particle size analyser	Delsa Nano common	Beckman Coulter, Inc.
12	Transmission electronic microscopy (TEM)	TECNAI G2-F20	Field Electron and Ion Co. Hillsboro
13	Spray dryer	Spray mate	JISL, India
14	Dissolution Apparatus	DS 8000	LabIndia, Thane west, Maharashtra, India
15	Borosilicate Tyype-I glass	NA	Tarson products Pvt. Ltd.,

			Kolkata, India
16	Refrigerator	WDE 205 CLS 3S	Whirlpool, India
17	Eppendorf tubes	2 ml	Tarson products Pvt. Ltd., Kolkata, India
18	Centrifuge	DS8000	LabIndia Analytical Instruments Pvt. Ltd., Navi Mumbai, Maharashtra
19	Stability chamber	CHM 105	REMI Electrotechnik Pvt Ltd. vasai, Mumbai, India
	Software Design Expert®	Version 10.0.1	Licenced to StatEase, USA (www. Statease.com)

CHAPTER 5
EXPERIMENTAL WORK

5.1 Physicochemical characterization of the drug

5.1.1 Physical appearance test

The organoleptic properties such as appearance, colour and odour of the drug Orlistat was observed and characterized.

5.1.2 Melting point

For the determination of melting point of Orlistat, the capillary method [USP 30 NF 25, 2007] was carried out in which the capillary tube was filled with drug up to 4mm at one sealed end. This capillary tube was then placed in the melting point apparatus and turned up the temperature and recorded the temperature at which the drug starts melting and the temperature at which the complete drug gets melted. Thus, the melting point range was observed.

5.1.3 Fourier transform infrared spectral analysis

The FTIR analysis of Orlistat was done by using the potassium bromide disk [Samyukta R.B. et al, 2011]. On the KBr-press, the pellets were made while applying 150 Kg/cm^2 of hydraulic pressure. These KBr pellets were then scanned under the FTIR, between the range of 4000 to 400 cm^{-1} wave number and the resulted spectra was compared with the standard FTIR specta of Orlistat to ensure the authenticity and purity of the drug.

5.2 Determination of absorbance maxima (λ_{max})

Accurately weighed 100 mg of Orlistat using digital weighing balance and then dissolved in small amount of methanol. This solution was poured into the 100 ml volumetric flask [Teja et al, 2015]. The volume was made up to 100 ml with methanol to give stock solution of 1mg/ml or 100 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. 0.5 ml of stock solution (1000 µg/ml) was transferred to 10 ml volumetric flask and the volume was made up by adding methanol. The resulting dilution was 50 µg/ml. Both the dilutions were scanned on a double beam UV-visible spectrophotometer. The wavelength at which maximum absorbance was shown by both the dilutions, that was recorded as λ_{max} for Orlistat.

5.3 Method validation for Orlistat in 0.5% Iodine solution in DCM

5.3.1 Calibration plot for Orlistat in 0.5% Iodine solution in DCM

100 mg of Orlistat was accurately weighed on calibrated digital weighing balance and was dissolved in small quantity of methanol. The solution was then transferred to 100 ml of volumetric flask. The volume was made up to 100 ml to give stock solution of 1mg/ml or 1000 µg/ml. Now from the stock solution, 0.04, 0.08, 0.12, 0.16 and 0.2 ml of solution was transferred into 10 ml volumetric flasks and added 1.2 ml 0.5% w/v iodine solution (prepared in DCM) to each volumetric flask. The mixture was kept aside for 20 minutes for complexation and then volume was made up to 10 ml with dichloromethane to form concentrations of 0.4, 0.8, 1.2, 0.16 and 2.0 µg/ml respectively [Teja et al, 2015]. The absorbance was noted at λ_{max} 368nm. The analysis was carried out in triplicate.

5.3.2 Linearity and Range

Linearity is the ability of the method to elicit the results of test samples 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 linearity is 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 50- 400 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 (0.4, 1.2 and 2.0) 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 were calculated.

5.3.4 Precision

The precision of an analytical procedure expresses the closeness 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 (0.4, 1.2, 2.0 µg/ml) covering the entire linearity range by intraday (repeatability) and interday studies (intermediate precision). Intraday precision was determined by analyzing (0.4, 1.2, 2.0 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 influence on solvent if handled by two different analysts. The % R.S.D was determined for solution of 0.4. 1.2 and 2.0 µg/ml by two different analysts.

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

Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value [ICH, Q2 (R1) guidelines, 2005]. The limit of quantification of an individual analytical procedure is the lowest 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 σ /S …….. (Equation 5.1)

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

(S= Slope of the regression equation)

L.O.Q = 10 σ /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 carried out for pure drug, excipients and drug: excipient mixture in ratio of 1:1. The above mixtures were placed in glass containers and stored at temperature 2-8 $^{\circ}$C and 50°C [ICH, Q1 A guidelines, 2005]. Observation of mixtures and pure samples were made on 0th and 15th day physically for color change, appearance, state and lump formation.

5.4.1.2 Chemical characterisation 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 Orlistat along with the excipients were observed. The effect of the excipients on the major peaks of Orlistat were observed to find the compatibility of the drug and excipients. The undisturbed peak of drug signifies compatibility of the drug with the excipients.

5.4.2 Solubility studies

The solubility study of Orlistat was done by using solvents that are used in the formulation to understand the solubility profile of drug [IP, 2014]. The different solutions *viz*., water, 0.1N HCl, dichloromethane, methanol, chloroform and lipid solution (GMS) were used in which the standard plots of Orlistat were recorded. 10 ml of each solvent was transferred to different containers to which, known excess amount of drug was added to saturate the solution. The drug solutions were kept on water bath shaker by maintaining the temperature 32 ± 2°C 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 368 nm. The drug concentration in each solvent was calculated from the standard plot and the graph was plotted between the concentrations vs. absorbance.

5.4.3 Partition coeffiecient

The partition coefficient study was performed by using octanol and water. Both the solvents (10 ml) were filled in glass container to which 10 mg of drug was added (excess amount) [Florey, 2008]. The mixture was allowed to shake for 24 hr at 37°C ± 2°C. 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 SLNs

METHOD 1 – Accurately weighed amount of GMS (Glyceryl mono stearate) was melted on water bath with soy lecithin by keeping at 60°C [Pooja *et al*., 2015] and then mixed into chloroform. Solution of excess amount of distilled water with PVA and tween 80, preheated at same temperature was added to molten GMS. The mixture was homogenized at 1200 rpm for 20 mins and then sonicated on bath sonicator for 40 minutes. The solvent was evaporated by spray drying to obtain the solid lipid nanoparticles.

METHOD 2 – Accurately weighed amount of GMS and soy lecithin were melted together on the water bath by keeping at 60°C [Pooja *et al*., 2015] and then mixed with chloroform. The mixture was added to the water, tween 80 and PVA solution (preheated at 70°C) using a syringe with needle no. 21 and stirring at 1800 rev/min for 30 mins. The solution was then sonicated for one hour to obtain a clear solution. The solvent was evaporated by spray drying to obtain the solid lipid nanoparticles.

METHOD 3 – Accurately weighed amount of GMS was melted with soy lecithin on water bath by keeping at 60°C and then mixed into chloroform. Preheated solution of water, tween 80 and PVA at same temperature was added to lipid phase using syringe and needle. The solution was homogenized at 1800 rpm for one hour and then sonicated on a bath sonicator for two hours to get the clear solution. The solvent was evaporated by spray drying to obtain the solid lipid nanoparticles.

To check the effect of lipid: polymer ratio on the properties of SLNs, different batches of SLNs were prepared as shown in table 5.1, by using the above mentioned methods for preparation of SLNs. Ten batches with low, medium and high ranges of GMS: PVA: chloroform were prepared. The effect of ratio was analyzed by observing the shape and size of the cubic particles on optical microscope at 100 X magnification.

5.5.2 Effect of preparation technique on SLN formulation

Table 5.4 presents the composition of SLN formulations prepared by using solvent evaporation technique [Pooja *et al*., 2015]. GMS and soy lecithin were weighed accurately and weighed amount of Orlistat was mixed with them. The ratios of the components were varied. The solid mixtures were molten in water bath by keeping the temperature at 60°C and

then mixed in to the organic solvent chloroform. Excess amount of distilled water (25 ml) with polymer PVA and surfactant Tween 80, preheated at the same temperature was added into the lipid phase using syringe and needle while stirring. The mixture was then homogenized at 1800 rpm for one hour. The obtained dispersion was then sonicated on a bath sonicator for two hours to reduce the size of the particles and get a clear solution. The solvent was evaporated by spray drying technique and obtained solid lipid nanoparticles in powdered form.

Table 5.1

Screening the ratio of components for formulations

Batch No.	**GMS : PVA : Chloroform**
A1	1 : 0.01 : 0.2
A2	1 : 0.015 : 0.25
A3	1 : 0.015 : 0.138
A4	1 : 0.01 : 0.04
A5	1 : 0.015 : 0.05
A6	1 : 0.02 : 0.15
A7	1 : 0.014 : 0.15
A8	1 : 0.01 : 0.077
A9	1 : 0.015 : 0.14
A10	1 : 0.02 : 0.125

Table 5.2

Preparation of solid lipid nanoparticles by solvent evaporation technique

S. No.	**Components**	**Range**
1.	Orlistat	0.13-0.47%
2.	Glyceryl monostearate	0.25%
3.	Chloroform	2.4- 48.0%
4.	Soy lecithin	0.75%
5.	Polyvinyl alcohol	2%
6	Tween 80	2%
7..	Water	25ml

5.6 Formulation Development trials

5.6.1 Preparation of optimized formulation by DoE technique

A Central composite design (*CCD*) was selected for four factors at three levels (X1, X2, X3 and X4) to optimize the response variables Y1 and Y2 respectively *i.e.* entrapment efficiency, *in-vitro* drug release at second hour. Design expert software was used for employing this design. Table 5.4 summarizes an account of seventeen 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 four 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 drug, organic solvent, duration of homogenization and duration of sonication. The translation of coded factors levels and value of variables is listed in table 5.3.

Table 5.3

Translation of experimental conditions into physical units

S. No.	Levels	Coded factors	(X1) Amount of Orlistat (%w/v)	(X2) Amount of Chloroform (%v/v)	(X3) Duration of Homogenization (min)	(X4) Duration of sonication (min)
1.	Low	-1	0.13	2.4	49	39
2.	Medium	0	0.3	26	75	90
3.	High	+1	0.47	48	100	140

5.7 Characterization and evaluation of SLNs

5.7.1 Optical microscopy

Optical microscopy was done by optical Microscope at 100 X using Oil immersion lens for viewing the abundance of solid lipid nanoparticle system and their physical appearance. The morphological characteristics were studied for SLN dispersion by optical microscopy. The photomicrographs of the preparations were obtained.

5.7.2 Transmission Electron Microscopy (TEM)

A drop of a sample was placed onto a carbon-coated grid and allowed to dry. The grid containing the sample was observed under the transmission electron microscope with an accelerating voltage of 120 kV. The nanoparticles were observed by focusing the lens. The

images were then obtained after focusing the microscope with different magnifications of 19000-29000 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 SLN formulation, which was prepared by single emulsification solvent evaporation technique. The particle size and the size distribution were observed and were reported for the optimized formulation.

5.7.4 Scanning Electron Microscopy

The physical appearance of the powdered solid lipid nanoparticle product of the optimized formulation was observed under the scanning electron microscope. A pinch of powdered SLN was placed under the microscope and get exposed to a focused beam of electrons which read the topography and composition of the sample and produced the images of the scanned surface of the SLNs.

Table 5.4

Factor combination as per experimental design

Run No.	(X1) Amount of Drug (%w/v)	(X2) Amount of organic solvent (%v/v)	(X3) Homogenizing time (mins)	(X4) Sonication time (mins)
F1	0.3	26	75	90
F2	0.4	40	90	60
F3	0.2	40	60	120
F4	0.2	12	60	60
F5	0.3	49.3	75	90
F6	0.5	12	90	60
F7	0.4	40	60	60
F8	0.4	12	60	120
F9	0.1318	26	75	90
F10	0.3	26	75	140.5
F11	0.468	26	75	90
F12	0.3	0.64	75	90
F13	0.3	26	100.2	90
F14	0.2	40	90	120
F15	0.4	12	90	120
F16	0.3	26	49.7	90
F17	0.3	26	75	39.5

Fig 5.1 Formulations prepared as per experimental design

5.7.5 Drug entrapment efficiency

Orlistat associated with SLNs was separated from unentrapped drug using centrifugation method [Jia *et al*., 2004]. SLNs were centrifuged at 15000 rpm for 45 mins at controlled temperature. Supernatant containing unentrapped Orlistat was withdrawn and measured by UV spectrophotometer at λmax 368 nm. The amount of Orlistat entrapped in SLNs was determined by calculating the entrapment efficiency as follows (equation 5.3):

$$EE\% = [A_t - A_f / A_t \times 100] \text{…….. (Equation 5.3)}$$

Where A_t is total amount of Orlistat and A_f is concentration of free Orlistat.

The entrapment efficiency was obtained by repeating the experiment in triplicate and the values were expressed as mean standard deviation.

5.7.6 In-vitro drug release

In vitro drug release of the suggested formulations was determined through diffusion membrane (mol. Size 12000- 14000 Da) [Ugaizo *et al*., 2002]. 2 ml SLN dispersion was filled in the membrane and the membrane was placed in the dissolution media of 150 ml 0.1 N HCl, 0.02% w/v tween 80 and 5% v/v methanol [Sateesha et al, 2011]. The temperature

was maintained at 32 °C ± 2 °C and stirring was set up to 100 rpm. 5 ml solution was extracted at out at the time interval of 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105 and 120 min and was replaced every time with fresh media to maintain sink condition. The absorbance of extracted samples was determined using UV spectrophotometer at 368 nm and the drug concentration present in the media was calculated by putting the values in to the standard equation.

5.8 Filling of prepared SLNs in capsules

The solid lipid nanoparticles dried by spray drying were filled in hard gelatin capsule shells along with the excipients. Lactose monohydrate was used as a diluent and 2% talc was used as a glidant [Vuddisa et al, 2014]. We obtained 5.8 g of SLN product from spray drying in which 1.2 g of drug was present. Orlistat has the doses of 120 mg and 60 mg in capsules. To prepare a 60 mg capsule we took 290 mg of SLN product and mixed it with excipients to fill the capsule. Table 5.5 shows the composition of the Orlistat SLN capsule. The capsules were filled manually using capsule filling machine.

Table 5.5

Composition of Orlistat SLN capsules

S. No.	Name of ingredient	Amount of ingredient (mg)
1.	Orlistat solid lipid nanoparticles formulation product	290
2.	Lactose monohydrate	215.8
3.	Talc	5.8

5.9 Evaluation of Orlistat SLN capsules

5.9.1 Weight variation

20 filled capsules were taken. Weighed one of the capsule on the digital balance and noted it reading. Then emptied the capsule completely and weighed the empty shell. By subtracting the filled capsule weight with empty capsule weight, the net weight of the content was determined. The process was repeated with all other capsules. The average of all the net weights was calculated. The percentage deviation of each capsule was calculated from the average net weight. The deviation should not cross the following limits shown in table 5.6.

Table 5.6

Limits of weight variation of capsules

Average net weight of capsule	Deviation (%)	No. of capsules
Less than 300 mg	± 10.0	Minimum 18
	± 20.0	Maximum 2
300 mg or more	± 7.5	Minimum 18
	± 15.0	Maximum 2

5.9.2 In-vitro dissolution study of Orlistat SLN capsule

In vitro dissolution study was performed on USP dissolution apparatus II [Mukund et al, 2016]. 900 ml of dissolution media, pH 1.2 0.1N HCl, 0.02% tween 80 and 5% methanol was filled in the vessels and both the capsules i.e. the prepared SLN capsule and the marketed capsule of Orlistat were put into their respective vessels. The rotations were set at 100 rpm and the temperature was maintained at 37 and 5ml of sample was extracted at the time interval of 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120 minutes. After every extraction, 5ml of fresh media was added in to the vessel to maintain sink condition. The samples were then filtered and observed under the UV spectrophotometer at 368 nm to determine their absorbance and concentration in the solution.

5.9.3 Stability study of SLN capsule

The stability study of SLNs were carried at different temperatures i.e. 25 ± 2°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 SLN capsule.

5.9.4 Analysis of Release Mechanism

In vitro release kinetics of Orlistat from SLN Capsule 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.*, first-order, Higuchi, Hixson-Crowell cube root, Korsemeyer-Peppas and zero-order 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. In table 5.7 various kinetic models with their equations are depicted.

Table 5.7

Mathematical release kinetic models for analyzing drug release

Model	Mathematical Equation	Expansion for abbreviations	Reference
Zero Order	$C=C_0-K_0t$	C = Amount of drug release, C_0 = Initial amount of drug in solution, K_0= Zero order rate constant, t = time	[Singhavi, 2011; Lokhandwala et al, 2013]
First Order	$logC=logC_o-Kt/2.303$	C = Amount of drug release, C_0=Initial concentration of drug K=First order constant, t=time	[Costa et al, 2001; Ramteke et al, 2014]
Higuchi Model	$C=[D(2qt-Cs)Cs\ t]^{1/2}$	C=Total amount of drug release per unit area of the matrix, D=diffusion coefficient for the drug in the matrix, qt=total amount of drug in a unit volume of matrix, Cs=dimensional solubility of drug in the polymer matrix, t=time	[Siepmann et al, 2012]
Hixson-Crowell Model	$C_0^{1/3}-C_t^{1/3}=KHC_t$	C_t=amount of drug released in time t, C_0 = Initial amount of drug, KHC=rate constant for Hixson-Crowell equation	[Dash et al, 2010]
Korsmeyer Peppas model	$Ct/C\infty= kt^n$	$Ct/C\infty$=fraction of drug release at time 't', k=rate constant, n=release exponent	[Lokhandwala et al, 2013]

CHAPTER 6

RESULTS AND DISCUSSION

6.1 Identification and characterization of itraconazole

6.1.1 Physical description

The sample of Orlistat 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 Orlistat

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 Orlistat

Parameter	Specification as per COA	Observation
Melting range	40-46 $^{\circ}$C	42°C

6.1.3 Identification of the drug Orlistat by FTIR spectra

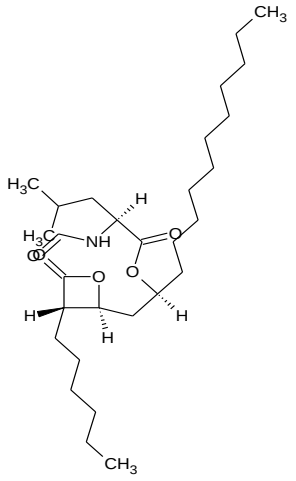


Fig. 6.1 Structure of Orlistat

The FTIR spectra of Orlistat exhibited many absorption bands at 2956.97, 2922.25, 2856.67 cm^{-1} representing the aldehyde C-H stretching. At 3333.1 cm^{-1} we can observe the N-H stretching, 1840.15 cm^{-1} shows C=O bond while 1670.41 and 1720.56 cm^{-1} displays the presence of cyclic C=O vibrations. The absorption band of 1464.02 cm^{-1} shows the aromatic C=C stretching and 1526.74 cm^{-1} is determined as the C-O bond. The identified functional groups with their estimated positions assure the presence of the drug (Venkateswarlu et al, 2016).

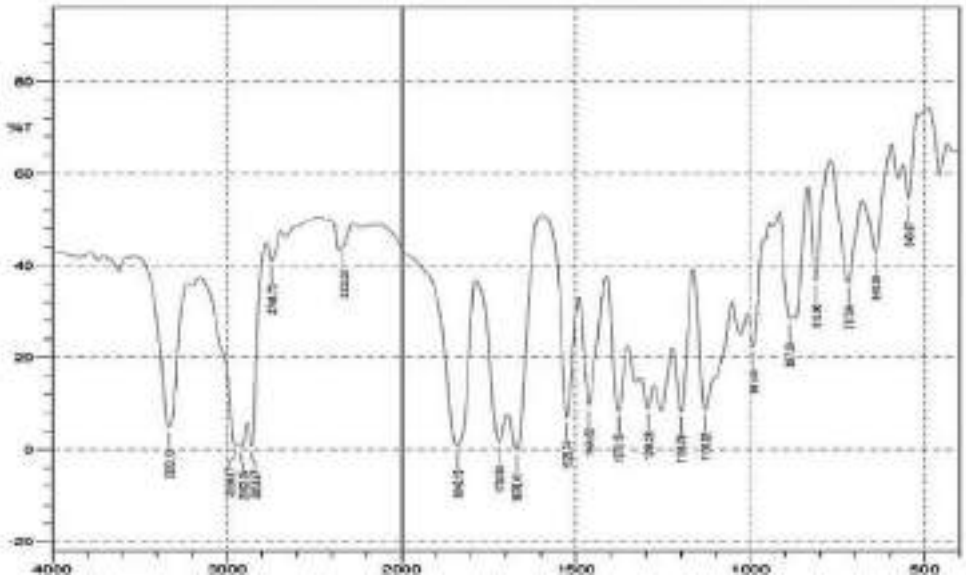


Fig 6.2 FTIR spectra of Orlistat

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

The λ_{max} of Orlistat was found to be 368 nm in 0.5% w/v Iodine solution in DCM. The scanning of the drug was done in the range (200-600 nm) as shown in the Fig.6.2.

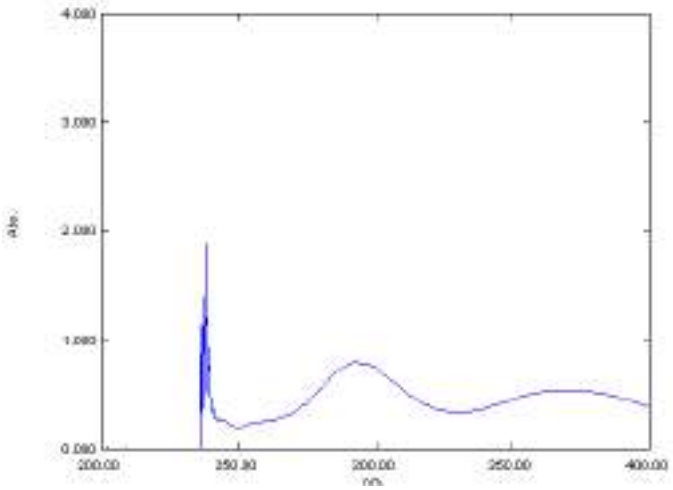


Fig. 6.3 Scan of Orlistat in 0.5% w/v Iodine solution in DCM when scanned between 200- 600 nm.

6.3 Analytical method validation of Orlistat in 0.5% w/v Iodine solution in DCM.

The U.V spectroscopic method was validated to check the suitability for the purpose prescribed (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 368 nm and the linearity was established in the range of 0.4-2.0 µg/ml with correlation coefficient, R^2= 0.9998. 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 Orlistat in 0.5% w/v Iodine solution in DCM.

The calibration plot of Orlistat was prepared by taking 0.4, 0.8, 1.2, 1.6, 2.0 µg/ml (table 6.3) concentrations of Orlistat in 0.5% w/v Iodine solution in DCM as shown in table 6.4. The experiments were performed in triplicate to find the standard deviation and percentage relative standard deviation. Absorbance range was found to be 0.147 - 0.745. The regression coefficient (R^2 value) was 0.9998 which showed linearity between 0.4-2.0 µg/ml concentrations. The Lambert Beer law was obeyed within the linearity range. The standard regression equation was found to be y = 0.3722 x + 0.001.

Table 6.3

Absorbance of Orlistat in 0.5% w/v Iodine solution in DCM at 368 nm.

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Relative Standard deviation(RSD)
0	0	0
0.4	0.147 ± 0.002	1.159
0.8	0.307 ± 0.004	1.219
1.2	0.445 ± 0.003	0.742
1.6	0.596 ± 0.003	0.481
2.0	0.745 ± 0.002	0.276
Linear Regression (R^2)= **0.9998**		

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.

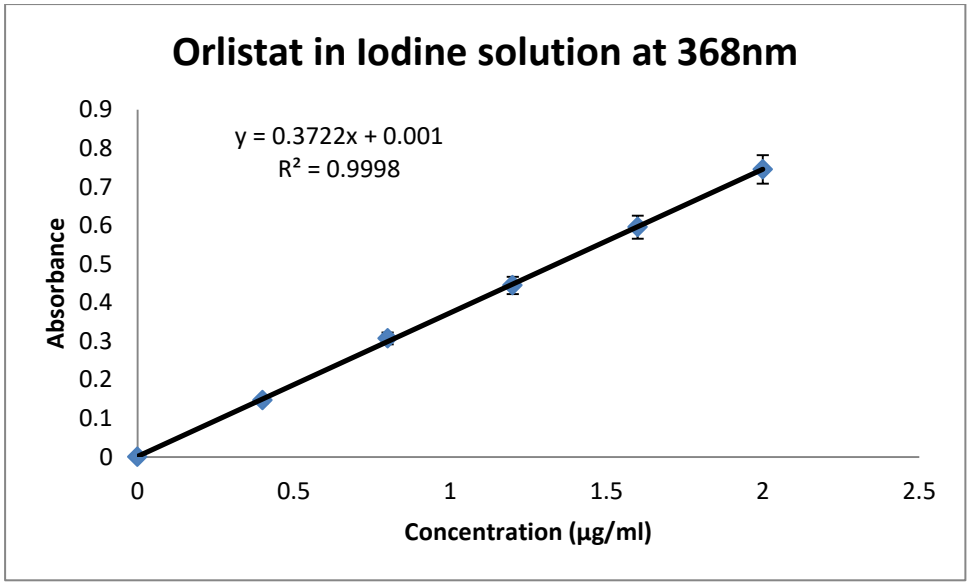


Fig. 6.4. Calibration curve of Orlistat in 0.5% w/v Iodine solution in DCM at 368 nm

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 97.9 – 99.2. These results proved that the method is accurate.

Table 6.4

Result of accuracy of Orlistat in 0.5% w/v Iodine solution in DCM.

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Mean recovery	% Relative Standard deviation(RSD)
0.4	0.392±0.002	97.9	0.52
1.2	1.191±0.003	99.2	0.28
2.0	1.973±0.012	98.6	0.62

6.3.4 Precision

The results of intraday, interday repeatability and reproducibility have been summarized in table 6.5 and 6.6 respectively. The results were found to show good reproducibility with % RSD below 2. The results were very close to the true value. There was negligible variation in intraday and interday precision. Percentage recovery of intraday precision was between 94.0-99.2 and interday precision was between 95.9-99.6.

Table 6.5

Result of intraday precision of Orlistat in 0.5% w/v Iodine solution in DCM.

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Mean recovery	% Relative Standard deviation(RSD)
0.4	0.376±0.007	94.0	1.74
1.2	1.191±0.006	99.2	0.48
2.0	1.984±0.005	99.2	0.27

Table 6.6

Result of interday precision of Orlistat in 0.5% w/v Iodine solution in DCM.

Concentration (µg/ml)	Mean Absorbance ± S.D (n= 3)	% Mean recovery	% Relative Standard deviation(RSD)
0.4	0.384±0.004	95.9	1.17
1.2	1.195±0.007	99.6	0.55
2.0	1.985±0.017	99.3	0.85

6.3.5 Robustness

The resulted robustness is shown in the table 6.7(1) and table 6.7(2). Both the absorbance taken by two different analysts using the same method displays almost same results, giving the % mean recovery falling into the range of 96.8-99.2 and 97.1-99.4 respectively. Also, the %RSD of both the analyst is below 2. Thus, it was found that the prepared method for validation of Orlistat is reliable.

Table 6.7

Analyst 1

Concentration (µg/ml)	Mean Absorbance ± S.D (n=3)	% Mean recovery	% Relative Standard deviation(RSD)
0.4	0.387 ± 0.003	96.8	0.85
1.2	1.191 ± 0.003	99.2	0.29
2.0	1.980 ± 0.012	99.0	0.60

Analyst 2

Concentration (µg/ml)	Mean Absorbance ± S.D (n=3)	% Mean recovery	% Relative Standard deviation(RSD)
0.4	0.388 ± 0.003	97.1	1.27
1.2	1.193 ± 0.003	99.4	0.14
2.0	1.980 ± 0.012	99.0	0.19

6.3.6 Limit of Detection and Limit of Quantification

The LOD and LOQ were found to be 0.104µg/ml and 0.352µg/ml respectively as shown in the table 6.8. Therefore, the drug can be detected between the above-mentioned range of concentrations.

Table 6.8
Characteristics for Orlistat in 0.5% w/v Iodine solution in DCM.

Parameters	Values
λmax (nm)	368
Linearity range (µg/ml)	0.4-2.0
Slope	0.3722
Intercept	0.001
Correlation coefficient (R^2)	0.9998
Accuracy (Percentage mean recovery)	97.9-99.2
Intraday Precision (Percentage mean recovery)	94.0-99.2
Interday Precision (Percentage mean recovery)	95.9-99.6
Robustness (Percentage mean recovery)	96.8-99.4
LOD (µg/ml)	0.104
LOQ (µg/ml)	0.352

6.4 Preformulation studies:

6.4.1 Drug excipient compatibility

Table 6.9
Compatibility studies of drug and excipients in 1:1 ratio

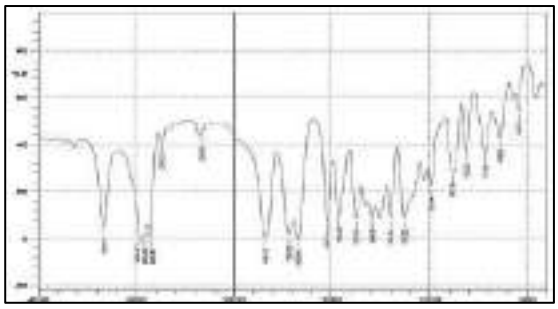
S.No.	Ingredients	Color	Appearance	State	Lumps
1.	Orlistat	White	Crystalline	Solid	Not Present
2.	GMS	White	Amorphous	Solid	Not Present
3.	PVA	White	Crystalline	Solid	Not Present
4.	Soy Lecithin	Yellowish brown	Amorphous	Solid	Not Present
5.	Orlistat : GMS	White	Crystalline	Solid	Not Present
6.	Orlistat : PVA	White	Crystalline	Solid	Not Present
7.	Orlistat : Soy Lecithin	Brownish white	Crystalline	Solid	Not Present
8.	Orlistat:PVA:GMS:Soy Lecithin	Brownish white	Crystalline	Solid	Not Present

1:1 ratio of drug and excipients observed at different time intervals

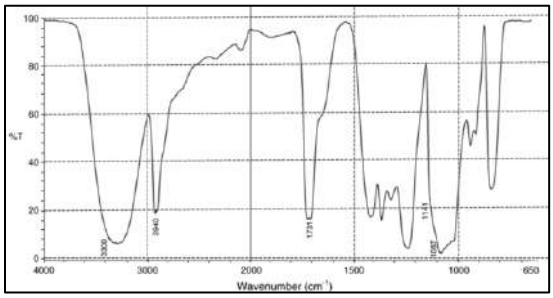
Ingredients	1st day	2nd day	3rd day	10th day	15th day
Orlistat					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
GMS (Glyceryl mono stearate)					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
PVA (Polyvinyl alcohol)					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Soy Lecithin					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	x	x
Orlistat : GMS					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Orlistat : PVA					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Orlistat : Soy lecithin					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	x	x
Orlistat:GMS:PVA:Soy lecithin					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	x	x

√ = No change occured ; x = Lumps were observed

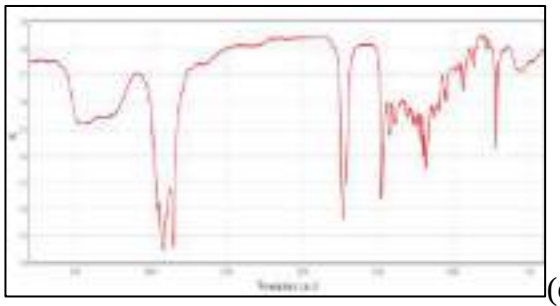
Fig 6.5 Drug-excipient compatibility study



(a) Drug

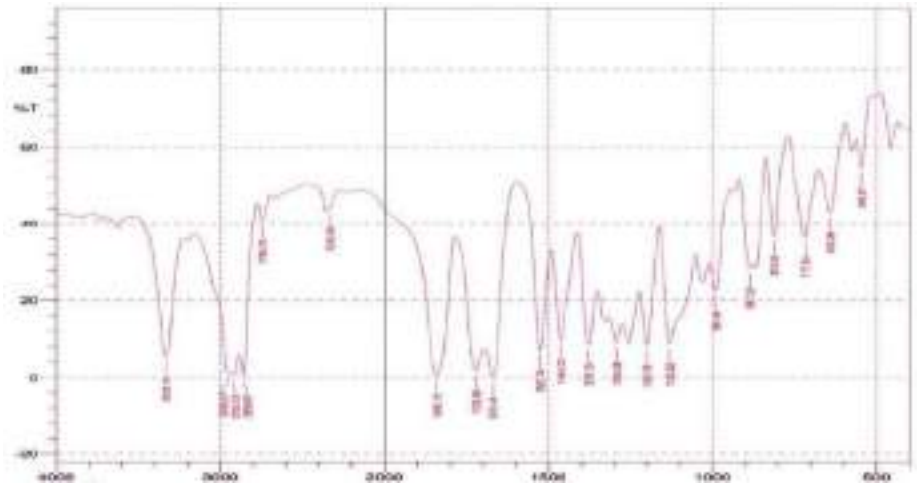


(b) Polymer

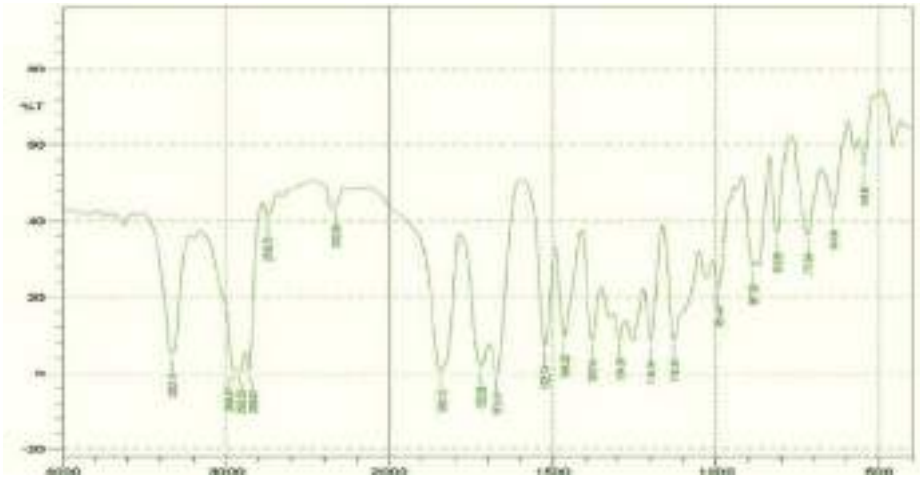


(c) Lipid

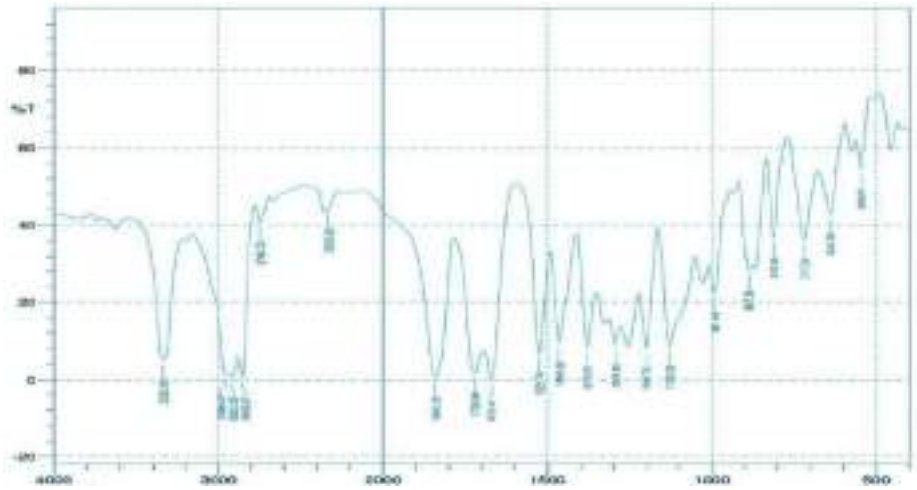
Fig 6.6 FT-IR spectra of (a) Drug (Orlistat) (b) Polymer (Polyvinyl alcohol) (c) Lipid (Glyceryl monostearate)



(a) Drug + Polymer



(b) Drug + Polymer + Lipid



(c) Drug + Polymer + Lipid + Stabilizer

Fig 6.7 FT-IR spectra of (a) Drug: Polymer, (b) Drug: Polymer: Lipid, (c) Drug: Polymer: Lipid: Stabilizer

6.4.2 Solubility analysis of Orlistat

The solubility data was obtained for Orlistat at 32°C using an ultraviolet absorption assay method to determine the concentration of drug present in the saturated solutions (IP, 2014). The solubility profile of drug with the buffers and lipid was helpful to determine that whether the drug was dispersed or solubilized in the water, organic solvents, lipid solution and buffer systems. The solubility profile in the decreasing order of solubility was found to be as follows: Water > 0.1 N HCl > Glyceryl monostearate > Dichloromethane > Chloroform > Methanol. The pH solubility profile of Orlistat was generated and was reported and shown in table 6.10. The solubility profile signifies that the drug gets freely solubilized in the lipid so it can be dispersed in the lipid system during the formulation. The dispensability can help in enhancing the pay load of drug in the SLN system. Thus, the solubility profile helped to generate the supportive information regarding the final formulation.

Table 6.10

Solubility profile of Orlistat in various solvents used in the formulation process (IP, 2014)

S.No.	Solvent	Solubility (mg/ml)	Solubility profile
1.	Water	0.03	Insoluble
2.	Dichloromethane	386	Freely soluble
3.	Methanol	1312	Very soluble
4.	Chloroform	472	Freely soluble
5.	Glyceryl monostearate	820	Freely soluble
6.	0.1 N HCl	0.22	Very slightly soluble

6.4.3 Partition coefficient of Orlistat

The partition coefficient of Orlistat was determined between water and octanol using shake flask method. It is also indicated as log P [Alex A et al, 1996]. The log P of Orlistat was found to be 2.8563.

6.4.4 Prescreening study for selection of ratio of components

Prescreening study was done to select the levels for design of experiment. For this, the formulations with suitable ratios of drug, PVA and chloroform keeping the lipid and water concentration constant were prepared. Levels were decided on the basis of literature. The SLNs were prepared by the single emulsification solvent evaporation technique and were

evaluated for various evaluation parameters. The ratio of the components was screened by optical microscopy as shown in table 6.11 and Fig.6.8.

Table 6.11

Ratio of the components screened by optical microscopy

Batch No.	Drug : PVA : Chloroform	Particles	Maximum size (nm)
A1	1 : 0.01 : 0.2	Present	1200
A2	1 : 0.015 : 0.25	Present	900
A3	1 : 0.015 : 0.138	Present	4700
A4	1 : 0.01 : 0.04	Present	500
A5	1 : 0.015 : 0.05	Present	3400
A6	1 : 0.02 : 0.15	Absent	-
A7	1 : 0.014 : 0.15	Present	1800
A8	1 : 0.01 : 0.077	Present	7200
A9	1 : 0.015 : 0.14	Absent	-
A10	1 : 0.02 : 0.125	Present	3720

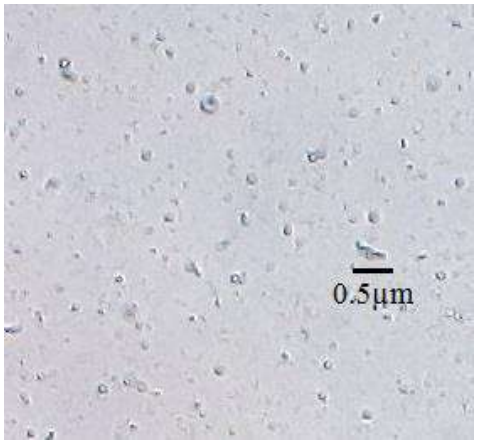


Fig 6.8 Optical photomicrograph representation of SLN preparation A4

6.5 Formulation development trials

6.5.1 Optimization of solid lipid nanoparticles by central composite design

The design of optimization contained four independent variables (X1, X2, X3, X4) and two dependent variables (Y1 and Y2). The X variables were drug (% w/v), organic solvent (% v/v) Homogenization time and Sonication time respectively, whereas, the Y variables were percentage entrapment efficiency and *in-vitro* % drug release. According to the design, 17 formulations were suggested. Each of them were formulated and analysed for two different

responses. The results were analysed by using polynomial modelling approach using the software, design expert. The responses were evaluated as given in the table 6.12

Table 6.12

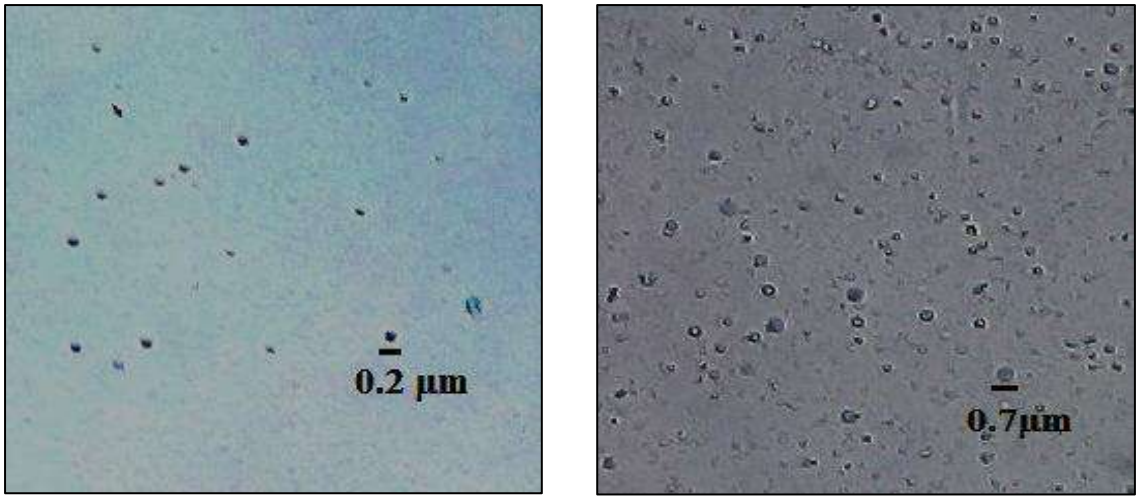
Factor combination and responses

Run No.	Amount of Drug (%w/v)	Amount of organic solvent (%v/v)	Homogenizing time (mins)	Sonication time (mins)	Entrapment efficiency (%)	*In-vitro* drug release (%)
F1	0.3	26	75	90	56.22	27.8
F2	0.4	40	90	60	24.68	43.2
F3	0.2	40	60	120	42.36	12.6
F4	0.2	12	60	60	88.08	69.7
F5	0.3	49.3	75	90	63.19	38.1
F6	0.5	12	90	60	93.77	87.6
F7	0.4	40	60	60	51.38	16.9
F8	0.4	12	60	120	53.28	52.0
F9	0.1318	26	75	90	10.87	41.8
F10	0.3	26	75	140.5	27.22	37.3
F11	0.468	26	75	90	23.76	62.2
F12	0.3	0.64	75	90	24.13	19.3
F13	0.3	26	100.2	90	64.71	26.7
F14	0.2	40	90	120	42.36	21.8
F15	0.4	12	90	120	61.48	11.3
F16	0.3	26	49.7	90	18.98	60.5
F17	0.3	26	75	39.5	21.24	45.4

6.6 Characterization and Evaluation of SLNs

6.6.1 Optical microscopy

The prepared formulations were examined for optical microscopy as shown in Fig.6.9. Optical microscopy showed that the spherical solid lipid nanoparticles were observed in formulations studied at 100 X.



(a) (b)

Fig 6.9 Optical micrograph representation of two of the formulations (a) Formulation F6 having particle size of 200 nm (b) Formulation F4 with 700 nm particle size

6.6.2 Transmission electron microscopy (TEM)

TEM photomicrographs of the representable solid lipid nanoparticles dispersion are shown in Fig 6.10 and Fig 6.11. The grid containing the sample was observed under the transmission electron microscope with an accelerating voltage of 120 kV with magnification between 190000 X –290000 X. The solid lipid nanoparticles were discrete and uniform. The diameter was found to be within the range of 70-200 nm.

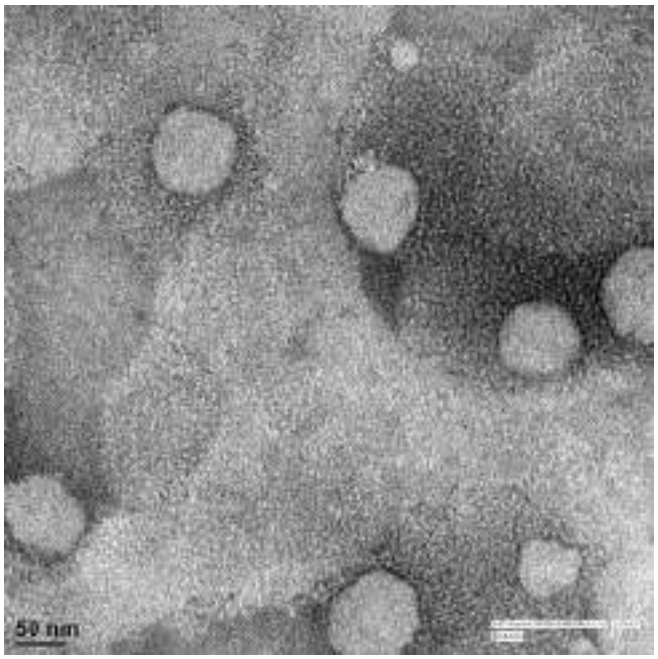


Fig 6.10 Transmission electron micrograph of SLN formulation F6 with the magnification of 290000X

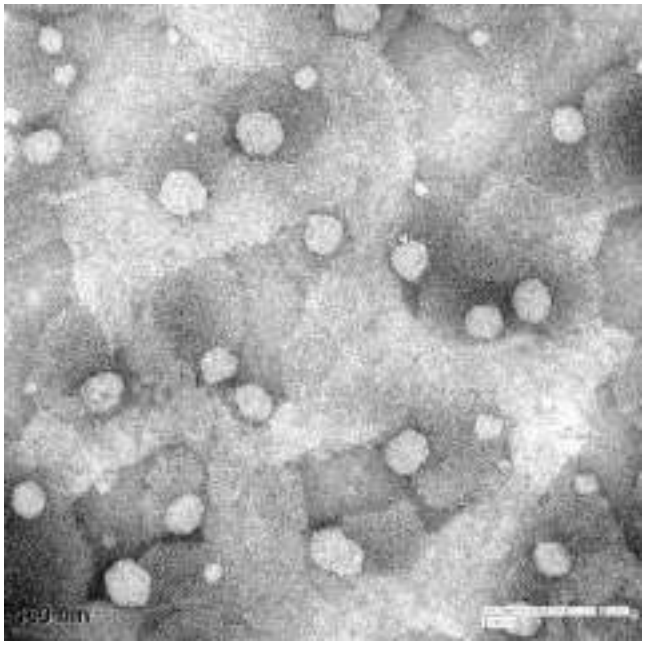
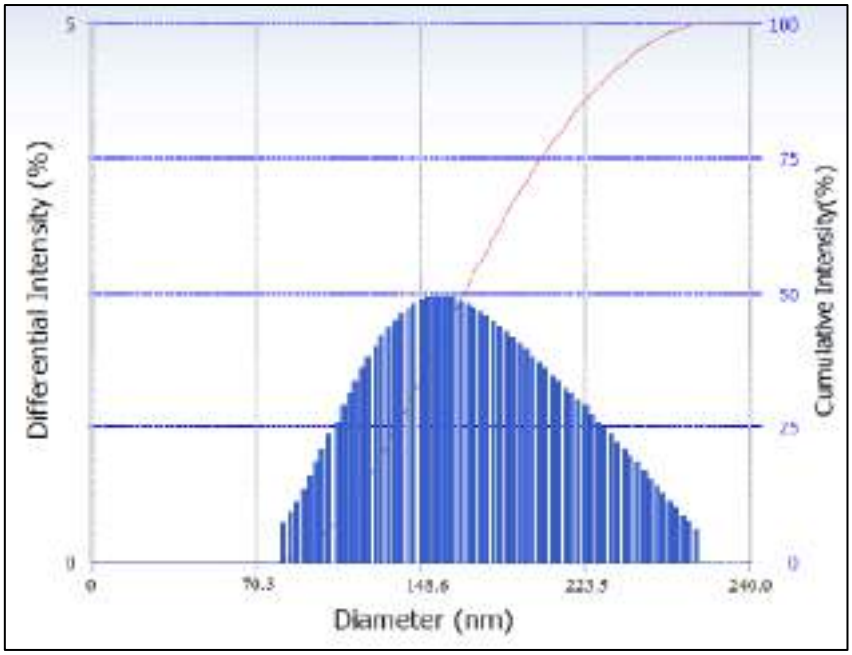


Fig 6.11 Transmission electron micrograph of SLN formulation F6 with the magnification of 190000X

6.6.3 Particle size analysis

The mean particle size of SLNs is presented in Fig 6.12. The differences in the particle size of SLN formulations prepared with variable ratios of lipid, stabilizer and drug were utilized to find the optimized formulation. The particle sizes were falling in the range of 70 - 240 nm as shown in Fig. 6.12. The optimized formulation showed average vesicle size of 212 nm with PI of 0.775. This shows that the optimized SLN formulation is homogeneous with uniform distribution.



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	95.0	39.1
2	212.9	9,939.4
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	152.4	9,871.2

Residual : 4.551e-003 (O.K)

Cumulants Results

Diameter	(d)	: 212.9	(nm)
Polydispersity Index	(P.I.)	: 0.775	
Diffusion Const.	(D)	: 1.813e-009	(cm^2/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 10760	(cps)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
30.0	0.0	0.0	224.0	0.0	11.5	172.1	1.4	20.4	283.8	2.2	77.3
32.5	0.0	0.0	242.7	0.0	11.5	112.2	1.5	21.9	252.1	2.2	79.5
35.2	0.0	0.0	263.1	0.0	11.5	163.9	1.6	23.5	262.0	2.1	81.5
38.2	0.0	0.0	185.1	0.0	11.5	128.3	1.7	25.3	209.7	2.0	83.5
41.4	0.3	0.3	109.0	0.0	11.5	106.6	1.8	27.1	220.2	1.9	85.4
44.8	0.4	0.7	134.8	0.0	11.5	199.7	1.9	29.1	262.1	1.8	87.1
48.6	0.5	1.2	162.9	0.0	11.5	109.0	2.0	31.1	224.8	1.7	88.8
52.7	0.6	1.8	193.2	0.0	11.5	135.8	2.1	33.2	218.3	1.6	90.4
57.1	0.6	2.4	126.2	0.0	11.5	181.7	2.2	35.5	253.7	1.4	91.8
61.9	0.7	3.0	161.9	0.0	11.5	148.1	2.3	37.8	242.7	1.3	93.1
67.0	0.7	3.7	100.5	0.0	11.5	136.8	2.4	40.1	298.2	1.2	94.3
72.7	0.7	4.5	142.4	0.0	11.5	249.7	2.4	42.6	234.3	1.1	95.4
78.7	0.7	5.2	187.9	0.0	11.5	288.8	2.5	45.0	265.9	1.0	96.4
85.3	0.7	6.0	137.1	0.0	11.5	256.3	2.5	47.6	209.6	0.9	97.3
92.5	0.7	6.7	190.4	0.0	11.5	254.6	2.5	50.1	283.0	0.8	98.0
100.2	0.7	7.4	148.2	0.3	11.8	286.2	2.6	52.7	205.3	0.6	98.7
108.6	0.7	8.1	110.9	0.4	12.2	254.0	2.6	55.3	197.5	0.5	99.2
117.7	0.6	8.8	178.8	0.5	12.7	260.9	2.6	57.8	182.1	0.4	99.6
127.6	0.6	9.4	152.4	0.6	13.2	210.3	2.6	60.4	183.6	0.4	100.0
138.2	0.5	9.9	102.1	0.7	13.9	205.6	2.5	63.0	128.6	0.0	100.0

D (10%) : 140.60 (nm) D (50%) : 136.80 (nm) D (90%) :221.30 (nm)

Fig 6.12 Particle size distribution of optimized SLN formulation (F6)

6.6.4 Scanning electron microscopy

The physical appearance of the powdered solid lipid nanoparticle product of the optimized formulation was observed under the scanning electron microscope. Fig. 6.13 shows the SEM image of Orlistat SLNs. The accelerating voltage was used 5 kV at the magnification of 18234X and 147887X.

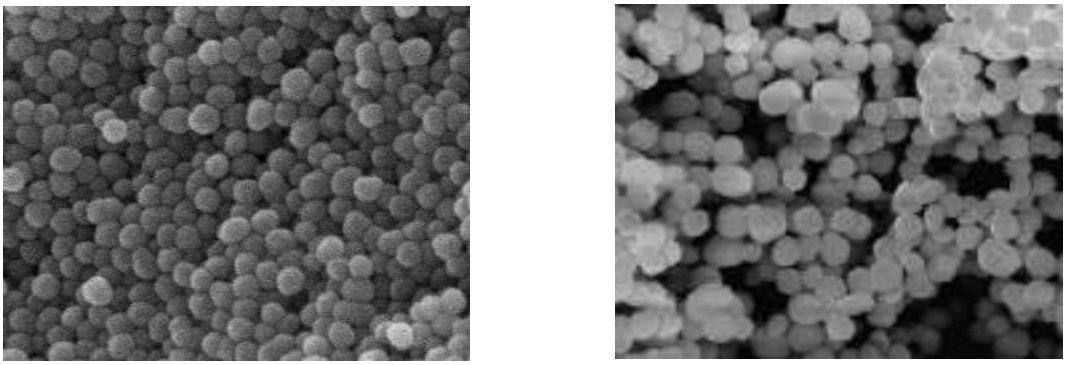


Fig. 6.13 SEM images of the optimized formulation of Orlistat SLN at the magnification of 18234X and 147887X respectively.

6.6.5 Entrapment efficiency

Table 6.13 represents the data for entrapment efficiency. Fig. 6.14 comparatively shows the entrapment efficiency of all the 17 formulations. From the entrapment data, it was observed that the ratio of the components within an optimum range offered good entrapment efficiency. The effect of the homogenization and sonication time was also determined from the study. When the formulation was treated with lesser amount of homogenizing and sonication time, it appeared as it was not able to form the desired nanoparticles and the size was rather big. It is possible that as the SLNs shows better drug entrapment, they also have effect on the drug loading. SLN formulation F6 shows maximum entrapment efficiency of 93.77%.

Table 6.13

% Entrapment efficiency of various solid lipid nanoparticle formulations:

Formulation	**Lipid : Drug : Organic solvent**	**Entrapment efficiency (%)**
F1	1 : 0.75 : 0.065	56.22
F2	1 : 1 : 0.1	24.68
F3	1 : 0.5 : 0.1	42.36
F4	1 : 0.5 : 0.03	88.08
F5	1 : 0.75 : 0.123	63.19
F6	1 : 0.5 : 0.03	93.77
F7	1 : 1 : 0.1	51.38
F8	1 : 1 : 0.03	53.28
F9	1 : 0.32 : 0.065	10.87
F10	1 : 0.75 : 0.065	27.22
F11	1 : 1.17 : 0.065	23.76
F12	1 : 0.75 : 0.0016	24.13
F13	1 : 0.75 : 0.065	62.71
F14	1 : 0.5 : 0.1	42.36
F15	1 : 1 : 0.03	61.48
F16	1 : 0.75 : 0.065	18.98
F17	1 : 0.75 : 0.065	21.24

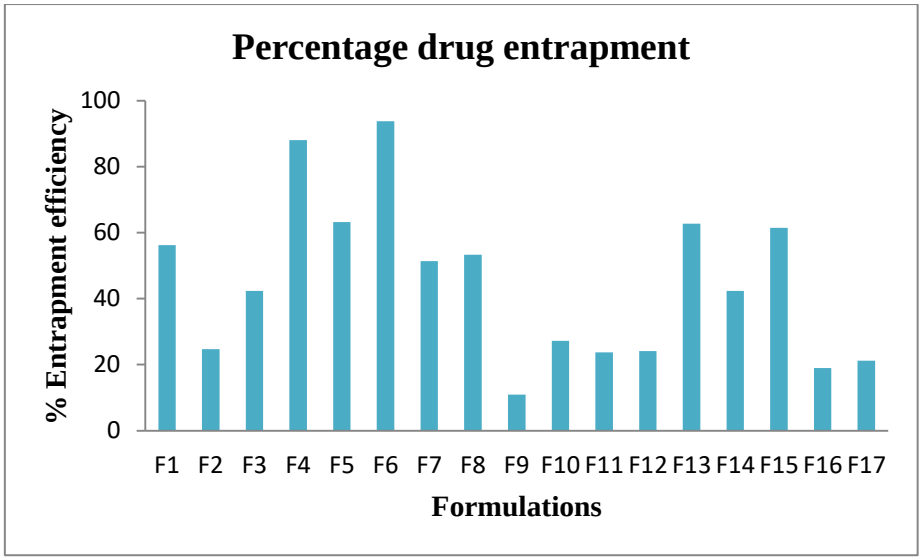


Fig 6.14 Percentage drug entrapment efficiency of various solid lipid nanoparticle formulations

6.6.6 Percentage in-vitro drug release

In vitro drug release of formulation through diffusion membrane (mol. Size 12000- 14000 Da) at second hour was done which was considered as one of the response in optimization study [Ugaizo *et al*., 2002]. The cumulative release of different formulation batches has been presented in Fig. 6.16. Table 6.14 and Fig.6.15 showed the percentage drug release data at 2nd hour. Drug release was found to be in range of 11.3 – 87.6 %. Generally, the drug release was found to increase within an optimum range of lipid and surfactant which could be due to the fact that the release of the drug through the torturous structure of solid lipid nanoparticles delays the drug release.

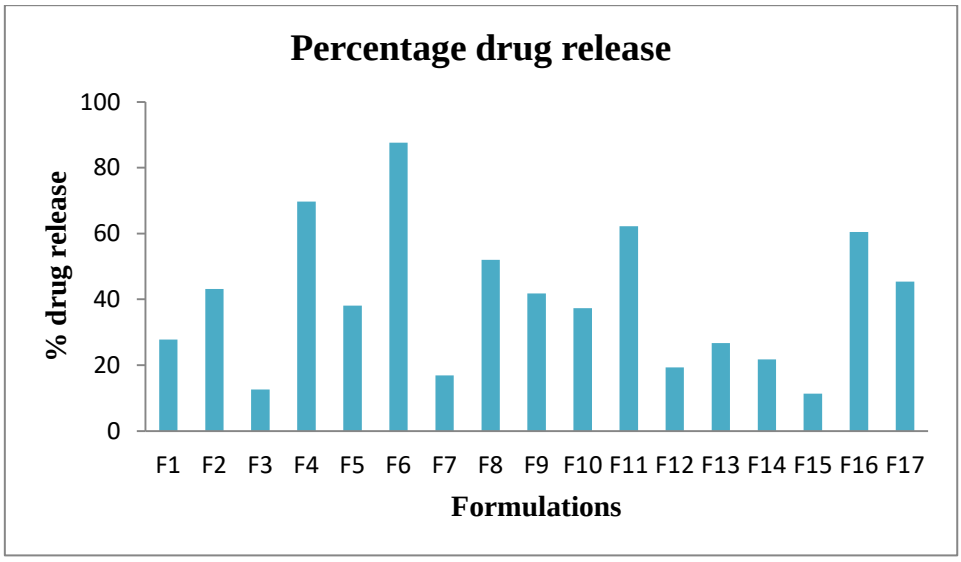


Fig 6.15 Percentage drug release profile of various solid lipid nanoparticle formulations

Table 6.14

% Drug release of various solid lipid nanoparticle formulations:

Formulation	Lipid : Drug : Organic solvent	% Drug Release
F1	1 : 0.75 : 0.065	27.8
F2	1 : 1 : 0.1	43.2
F3	1 : 0.5 : 0.1	12.6
F4	1 : 0.5 : 0.03	69.7
F5	1 : 0.75 : 0.123	38.1
F6	1 : 0.5 : 0.03	87.6
F7	1 : 1 : 0.1	16.9
F8	1 : 1 : 0.03	52.0
F9	1 : 0.32 : 0.065	41.8
F10	1 : 0.75 : 0.065	37.3
F11	1 : 1.17 : 0.065	62.2
F12	1 : 0.75 : 0.0016	19.3
F13	1 : 0.75 : 0.065	26.7
F14	1 : 0.5 : 0.1	21.8
F15	1 : 1 : 0.03	11.3
F16	1 : 0.75 : 0.065	60.5
F17	1 : 0.75 : 0.065	45.4

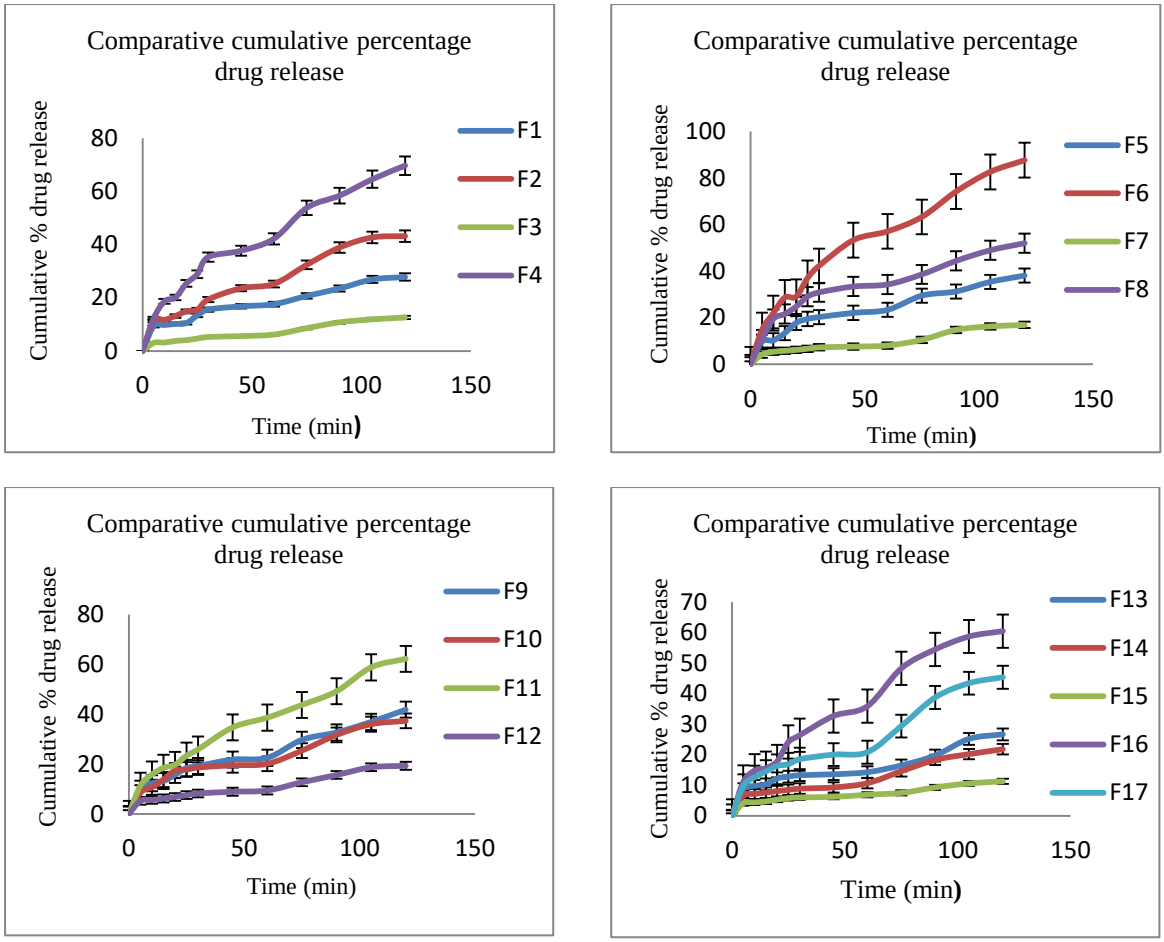


Fig 6.16 Comparative cumulative % drug release profile of different SLN formulations

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 (Orlistat), organic solvent (Chloroform). A Central Composite Response Surface Rotatable Design was employed to obtain 17 different factor combinations and replicates where two independent variables were studied at three levels (Stat-Ease, 2017). Different factor combinations that were obtained and experimentally run to measure the responses *Y1* (percent entrapment efficiency) and *Y2* (percent drug release) are given in table 6.15.

Figure 6.17 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 0.67 which means that fraction of design space capable of predicting the true average within 1, standard deviation was 85%, which is higher than the recommended 80% value.

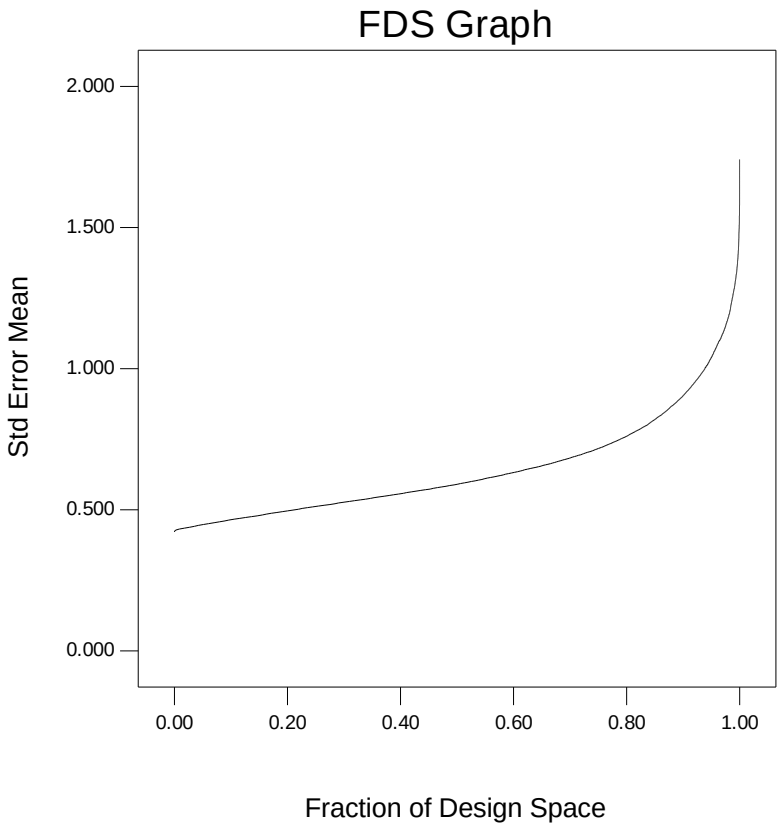


Fig. 6.17 Fraction of design space (FDS) graph for mean standard error

6.7.1 Statistical analysis

The formulations prepared according to the design were analyzed by using Design Expert® ver 10.0.6.0 software package. The effect of formulation variables on the response variables were statistically evaluated by one way ANOVA at 0.05 levels [Stat-Ease 2017]. The design was evaluated by response surface method using following polynomial equation 6.1:

$$Y=\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 \ldots\ldots\ldots \text{(Equation 6.1)}$$

where, Y is the response variable, β_0 the constant and $\beta_1, \beta_2, \beta_3$ are the regression coefficients. X_1 and X_2 stand for the main effect, X_1X_2 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 release were set in the range of 10 – 90.

In order to determine the significant design terms, their interactions and their effect on the response variables Y_1 and Y_2, 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 R^2 and predicted R^2. ANOVA was used to generate the quadratic (Y_1 and Y_3) or linear (Y_2) polynomial model equations. The model values are given in table 6.15.

The models were found suitable for the response variables Y_1 and Y_2 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 (0.9843, 0.9839, 0.9746) and predicted R^2 (0.9621, 0.9773, 0.9255) to actual model R^2 (0.9921, 0.9869 and 0.9873) also indicated the goodness of fit to the data. The observed values of R^2 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 37.62 to 52.318, 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 four 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 lipid, stabilizer and drug, 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:

Entrapment efficiency= +47.27 + 3.83 * A + 11.61 * B + 4.69 * C + 1.78 * D + 9.08 * AB - 3.02 * AC + 28.59 * AD – 5.07 * BC + 13.30 * BD + 3.65 *
CD…….. (Equation 6.2)

Drug release = +37.40 + 6.06 * A + 5.59 * B – 3.23 * C – 2.41 *D + 12.55 * AB – 5.19 * AC + 21.35 * AD + 7.29 * BC + 14.60 * BD – 9.46 * CD……(Equation 6.3)

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

Entrapment efficiency = - 329.72648 – 3.34756 * Drug – 8.61897 * Chloroform + 0.81558 * Homogenization – 4.23181 * Sonication + 0.10379 * Drug * Chloroform – 8.06333E-003 * Drug * Homogenization + 0.038122 * Drug * Sonication – 0.096643 * Chloroform * Homogenization + 0.12668 * Chloroform * Sonication + 8.11389E-003 * Homogenization * Sonication……..(Equation 6.4)

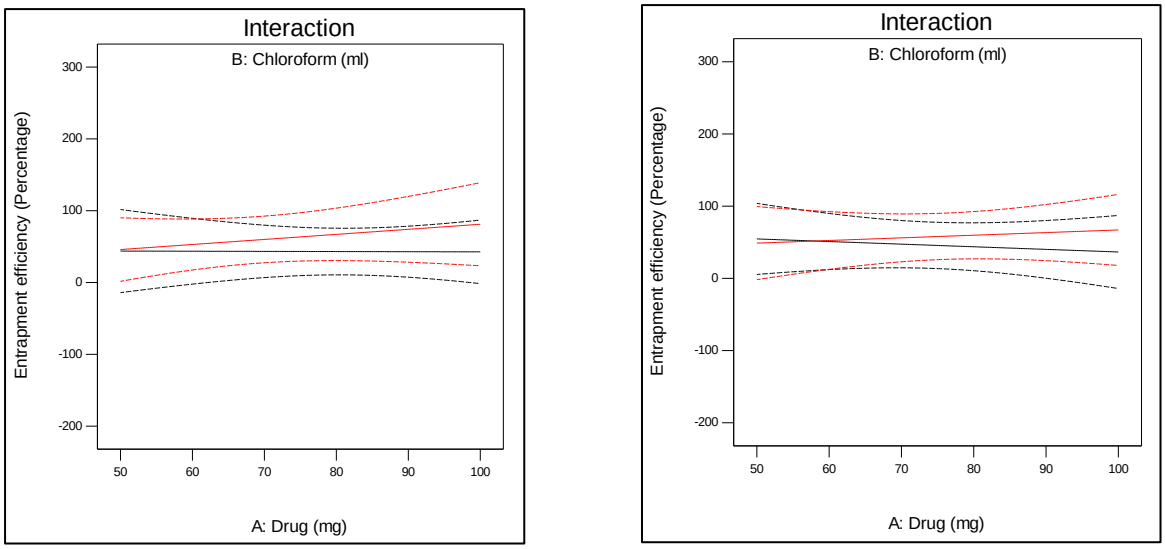
Drug release = + 223.59939 – 2.21472 * Drug – 32.09105 * Chloroform + 1.81224 * Homogenization – 1.54233 * Sonication + 0.14348 * Drug * Chloroform – 0.013833 * Drug * Homogenization + 0.028469 * Drug * Sonication + 0.13881 * Chloroform * Homogenization + 0.13907 * Chloroform * Sonication – 0.021028 * Homogenization * Sonication ……. …….....(Equation 6.5)

Response surface graphs, interaction plots and perturbation plots for entrapment efficiency and percentage drug release as per CCD were obtained from Design Expert® software. Fig. 6.18, 6.19 and 6.20 showed interaction plots, perturbation plots and contour plots for entrapment efficiency respectively. In the same way, Fig. 6.21, 6.22 and 6.23 represented interaction plots, perturbation plots and contour plots for percentage drug release.

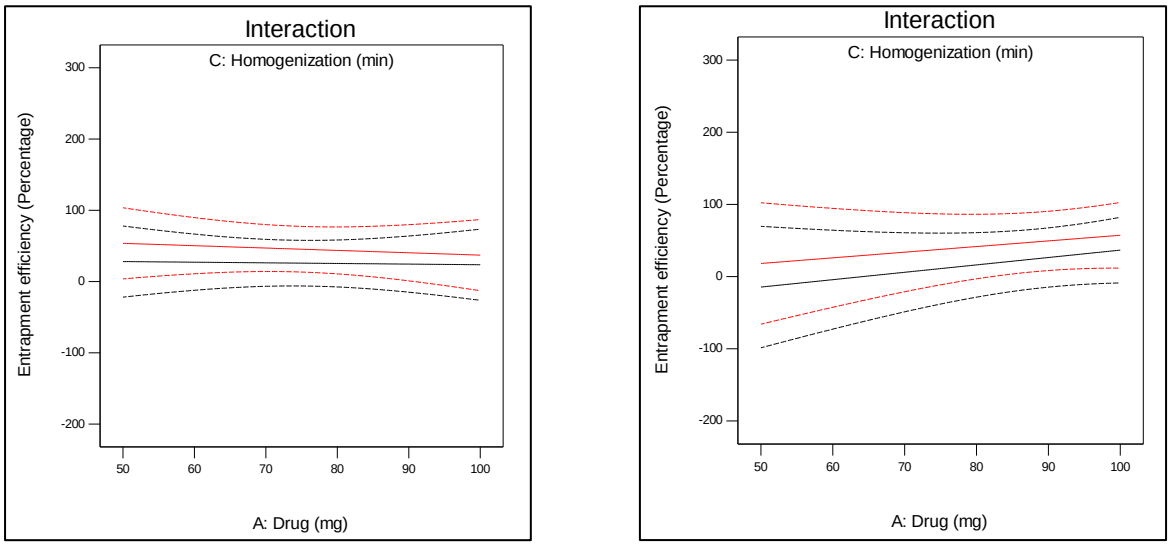
Table 6.15

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

Coefficient (Factors)	**Entrapment efficiency *(Y_1)***	**% Drug release *(Y_2)***
Intercept	47.27 *(p=0.4865)*	37.40 *(p=0.0221)*
A-Drug	3.83 *(p=0.6890)*	6.06 *(p=0.2845)*
B-Chloroform	11.61 *(p=0.2402)*	5.59 *(p=0.3218)*
C-Homogenization	4.69 *(p=0.4511)*	-3.23 *(p=0.3711)*
D-Sonication	1.78 *(p=0.8522)*	-2.41 *(p=0.6630)*
AB	9.08 *(p=0.4720)*	12.55 *(p=0.1034)*
AC	-3.02 *(p=0.7071)*	-5.19 *(p=0.2768)*
AD	28.59 *(p=0.0404)*	21.35 *(p=0.0123)*
BC	-5.07 *(p=0.5311)*	7.29 *(p=0.1372)*
BD	13.30 *(p=0.2993)*	14.60 *(p=0.0638)*
CD	3.65 *(p=0.6506)*	-9.46 *(p=0.0623)*
R^2	0.5055	0.7942
Adj. R^2	0.0110	0.5884
Pred. R^2	-7.2399	-1.1669
Adeq. Precision	4.316	8.265

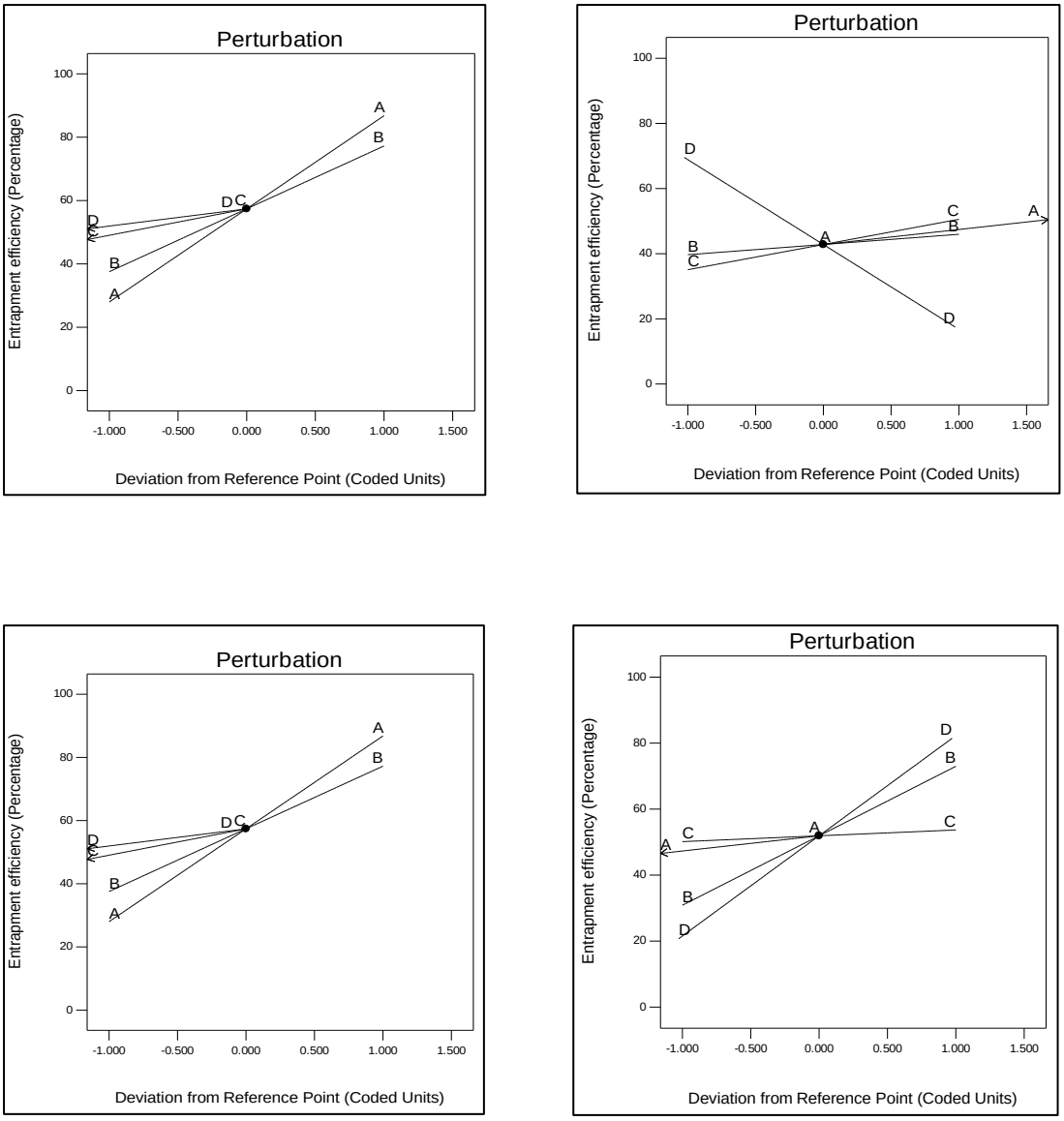


(a)



(b)

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



(a) (b)

Fig. 6.19 (a) Perturbation plots for the terms B and C with respect to response variable Y1 while the term A (Drug) is constant at all levels of B and C (b) Perturbation plots for the terms A and C with respect to response variable Y1 while the term B (Organic solvent) is constant at all levels of A and C

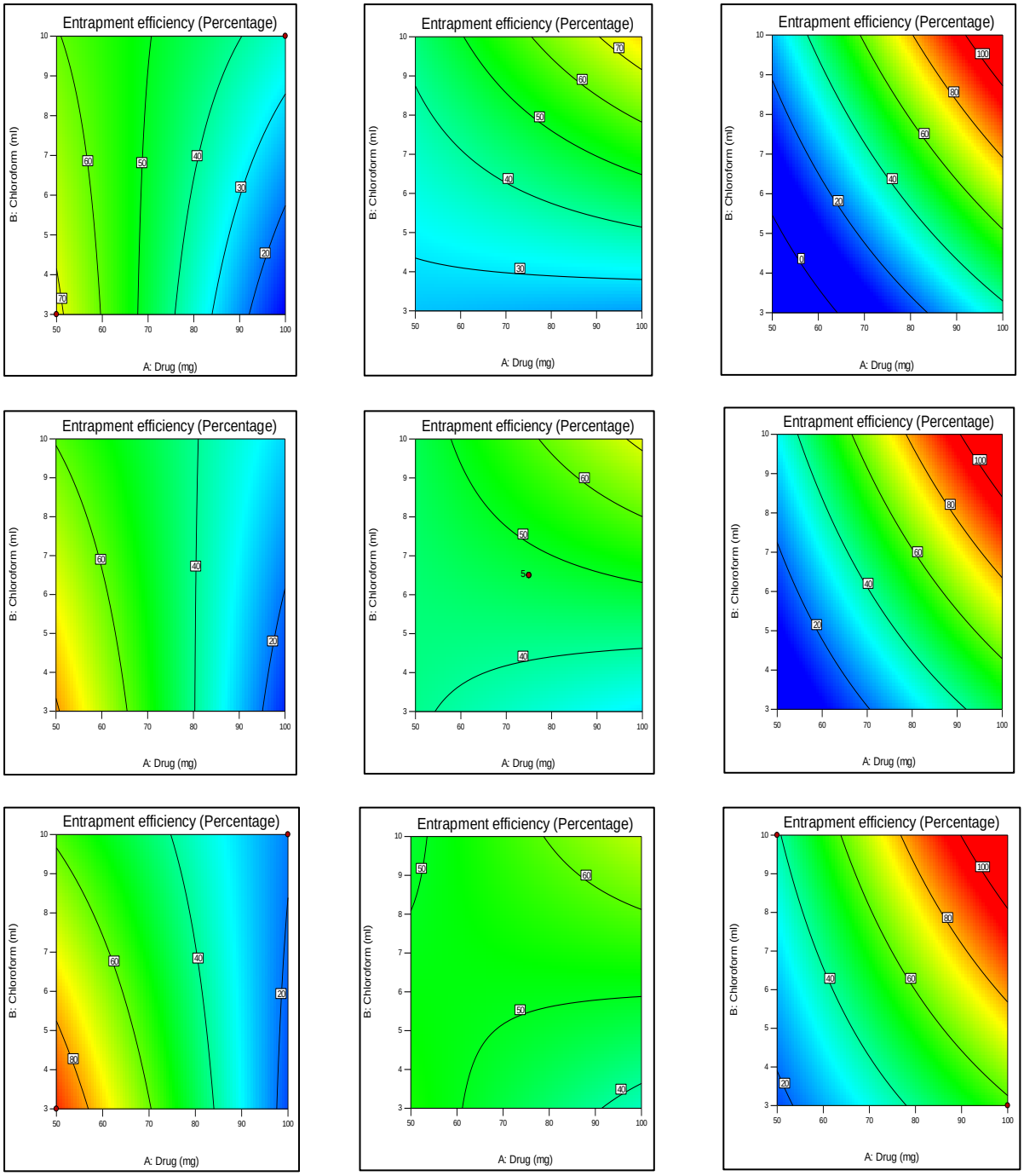
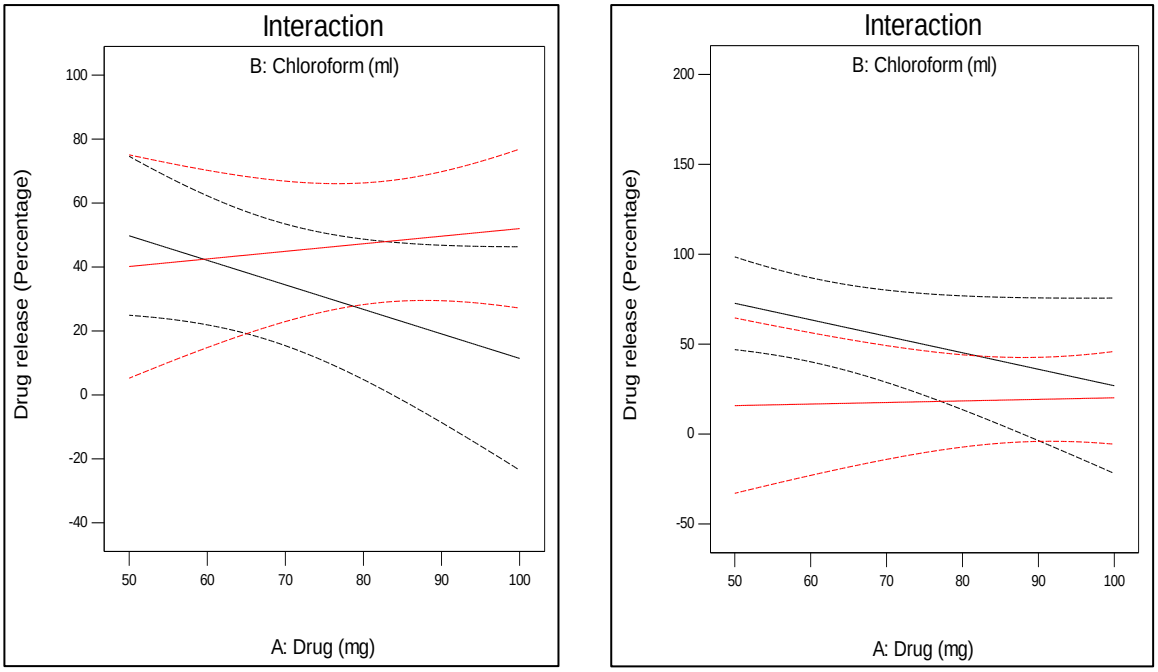
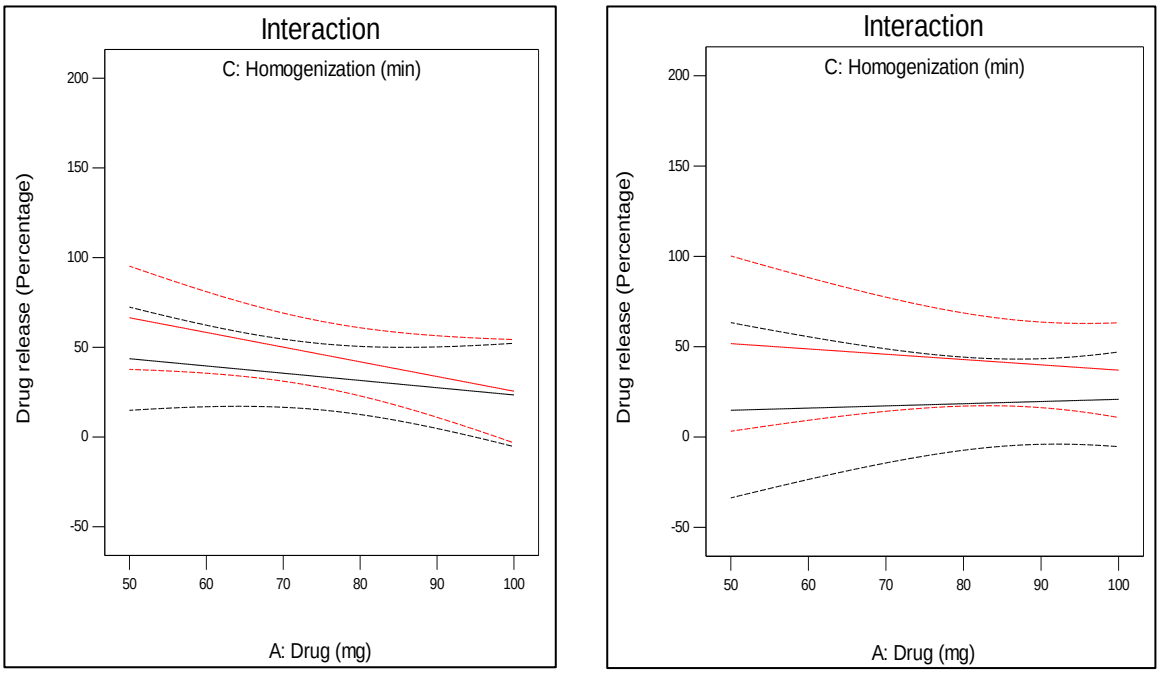


Fig 6.20 Representative contour plots for terms A (Drug) and B (chloroform) with respect to response variable Y1 (Entrapment efficiency) at all different levels of homogenization and sonication.

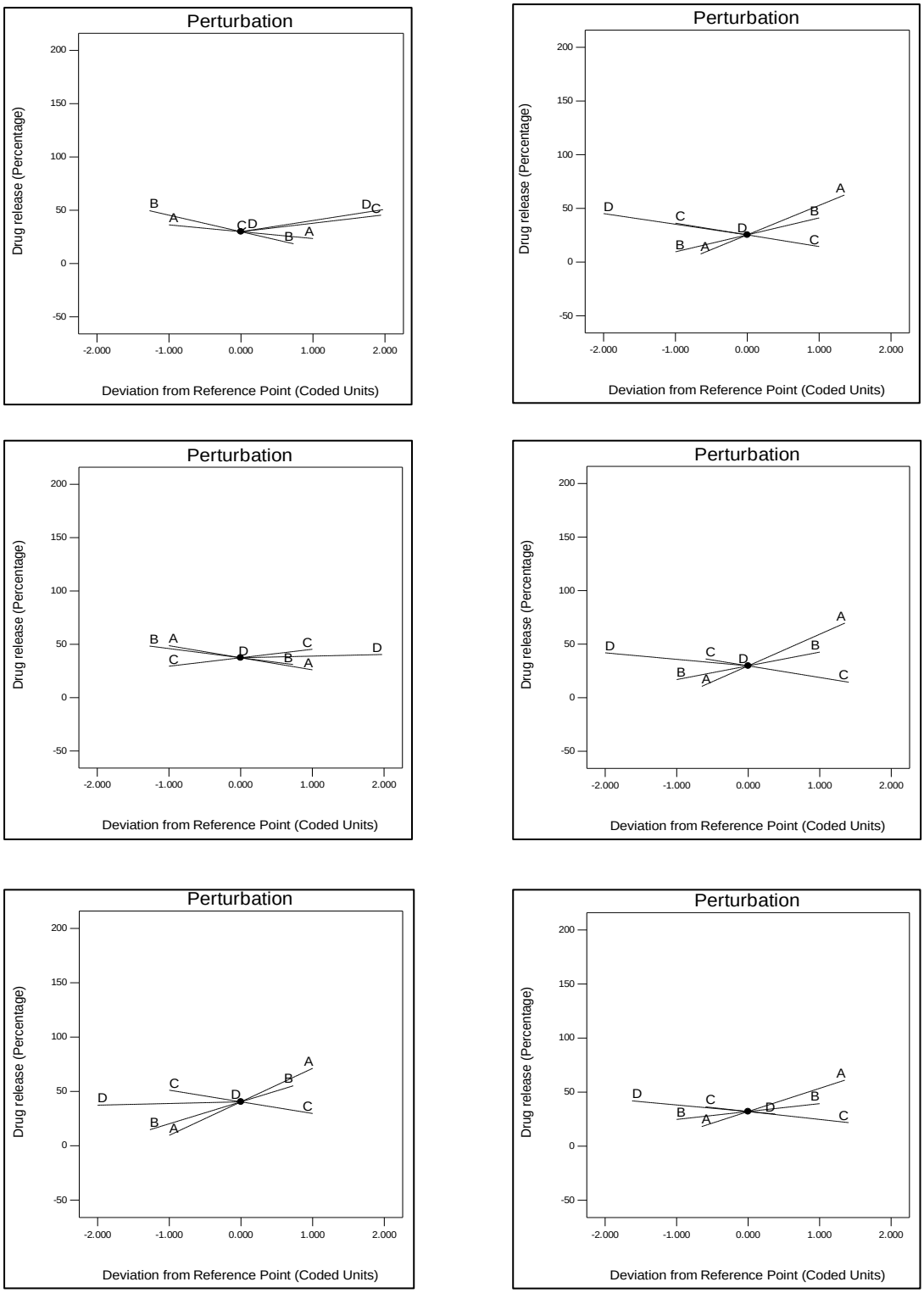


(a)



(b)

Fig. 6.21. (a) Interaction plots for the terms A and B with respect to response variable Y_2 while the term C is constant at all levels of A and B (b) Interaction plots for the terms A and C with respect to response variable Y_2 while the term B is constant at all levels of A and B



(a) (b)

Fig 6.22 (a) Perturbation plots for the terms B and C with respect to response variable Y_2 while the term A (Drug) is constant at all levels of B and C (b) Perturbation plots for the terms A and C with respect to response variable Y_2 while the term B (Organic solvent) is constant at all levels of A and C.

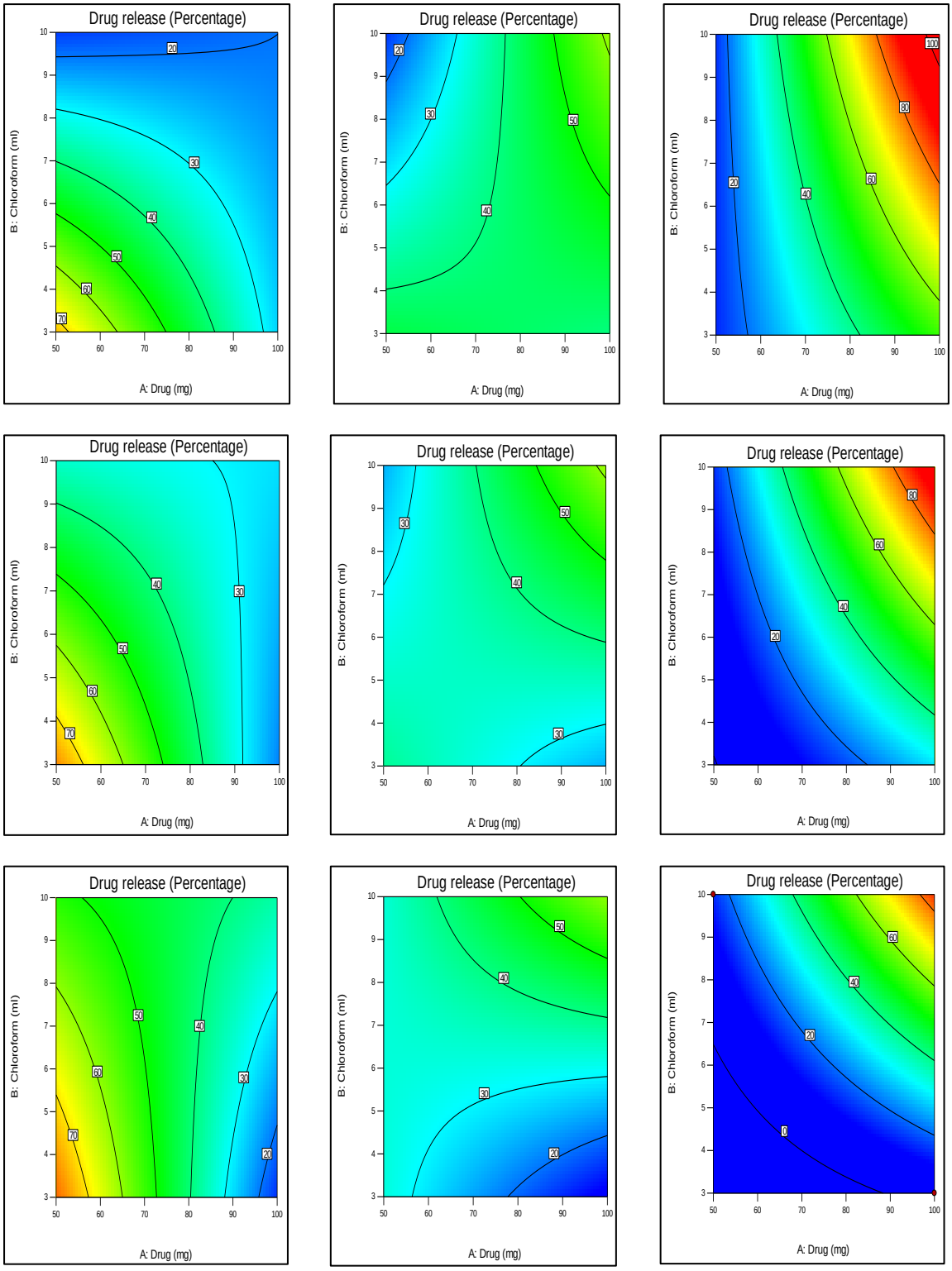


Fig 6.23 Representative contour plots for terms A (Drug) and B (chloroform) with respect to response variable Y2 (*In-vitro* % drug release) at all different levels of homogenization and sonication.

6.7.2 Validation of Optimized results

The optimization design suggested hundred best optimized batches after processing, out of which best two were considered as shown in table 6.16. Comparison of experimental and predicted responses, *i.e.* entrapment efficiency and drug permeation as per DoE along with percentage error is listed in table 6.17. The percentage error ranged between -8.43 and 9.62 (table 6.17). These data showed that most of the predicted values are close to the experimental values. These indicated the prognostic ability of the SLN formulation of Orlistat 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 6.16

Validation of optimized batch of SLN dispersion

Formulation Code	Drug (mg)	Chloroform (ml)	Homogenization time (min)	Sonication time (min)
V1	50	3	90	60
V2	94.009	9.767	61.659	116.862

Table 6.17

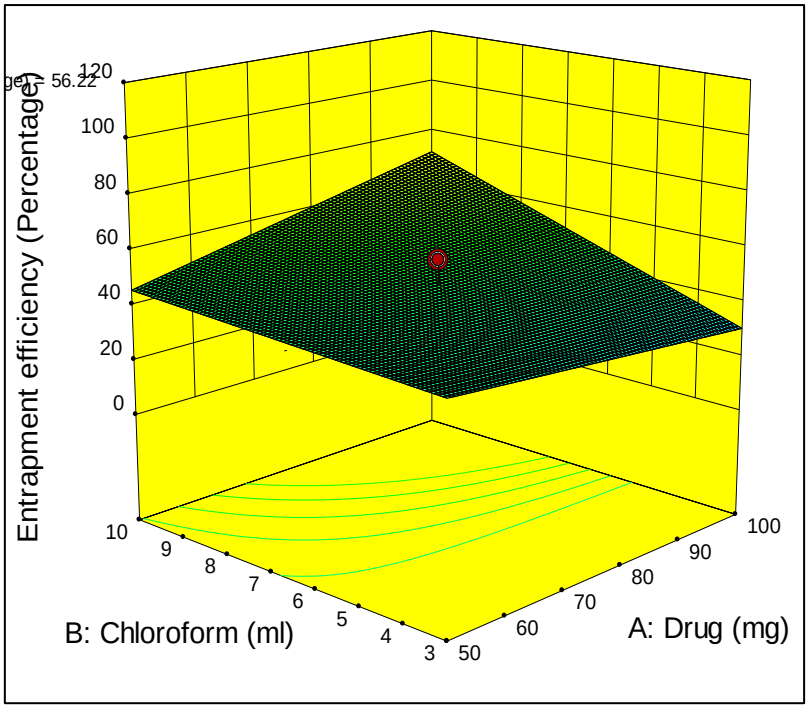
Comparison of Experimental results with predicted values with percentage error

Formulation Code	Response	Predicted values	Experimental values	Percentage error
V1	Entrapment efficiency (%)	90.157	93.7	-3.93
	Drug release (%)	80.793	87.6	-8.43
V2	Entrapment efficiency (%)	98.809	89.3	9.62
	Drug release (%)	88.589	85.4	3.59

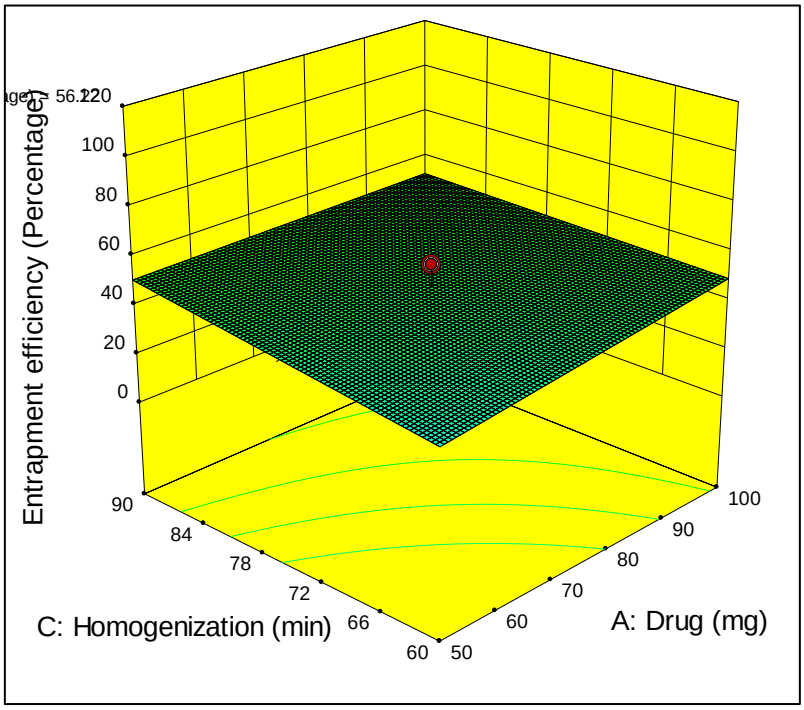
The optimized and validated SLN dispersion V1 with the composition as shown in table 6.16 was analyzed on the basis of statistical parameters. 3-D plots for entrapment efficiency and drug release as shown in Fig. 6.23 and 6.24 respectively, represents the optimized range of the components, GMS, chloroform, homogenizing time and sonication time, which can

provide the best possible entrapment efficiency and % drug release in combination. The plots help to define the relationship between the components by observing the response surface. The optimized SLN dispersion offered percentage entrapment efficiency of 93.7 % and percentage drug release of 87.6 %. The observed responses were the best optimal responses in combination as compared to other suggested responses.

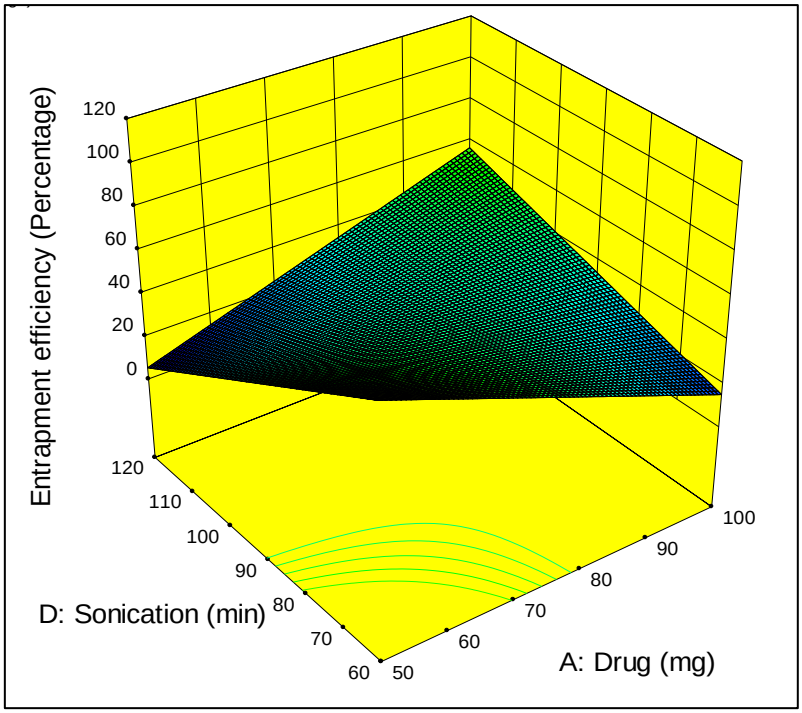
The 3-D plots along clearly defined the optimal range of the SLN formulation. The optimized formulation was found to offer the best optimal responses in the form of percentage entrapment efficiency and percentage drug release. The repeatability and robustness of the preparation of SLN dispersion and 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.


Entrapment efficiency (Percentage)
56.22
120
100
80
60
40
20
0
10
9
8
7
6
5
4
3
B: Chloroform (ml)
50
60
70
80
90
100
A: Drug (mg)

(a)

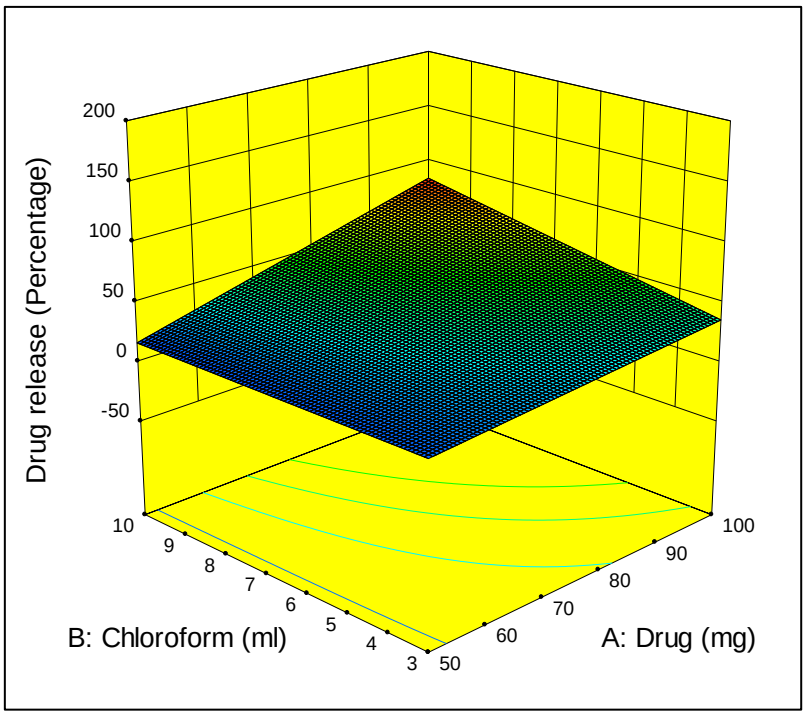

Entrapment efficiency (Percentage)
56.22
120
100
80
60
40
20
0
90
84
78
72
66
60
C: Homogenization (min)
50
60
70
80
90
100
A: Drug (mg)

(b)

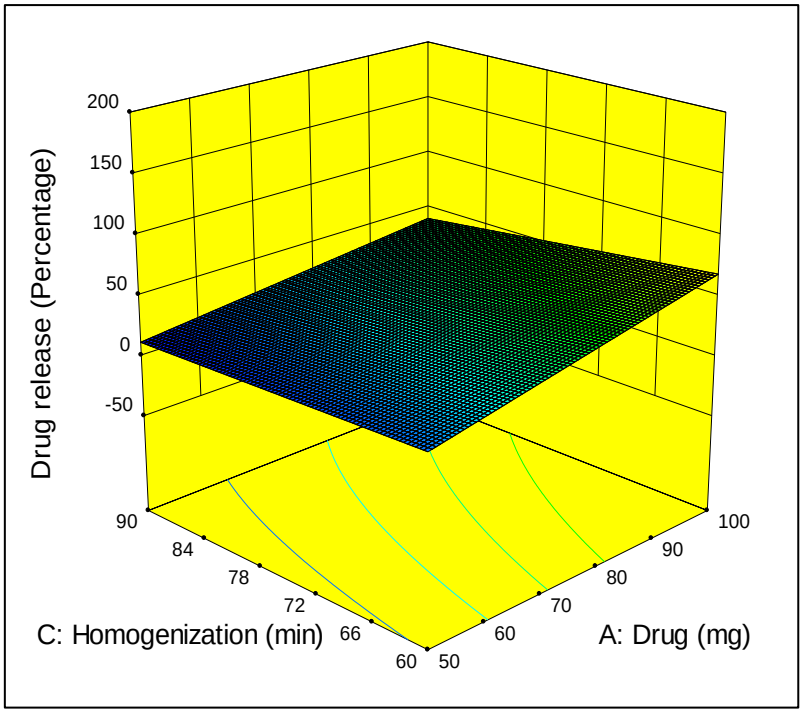


(c)

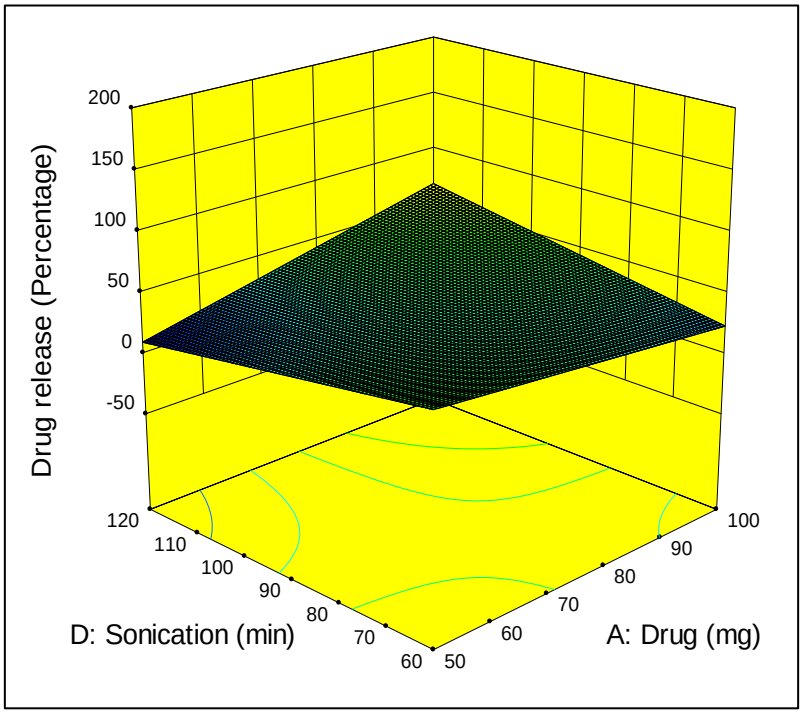
Fig 6.24 3-D plots of optimized SLN formulation (V1) for Entrapment efficiency (a) Plot between drug and chloroform keeping X3 and X4 constant, (b) Plot between Drug and homogenization keeping chloroform and X4 constant, (c) Plot between Drug and sonication time keeping chloroform and X3 constant.



(a)



(b)



(c)

Fig 6.25 3-D plots of optimized SLN formulation (V1) for percentage drug release (a) Plot between drug and chloroform keeping X3 and X4 constant, (b) Plot between Drug and homogenization keeping chloroform and X4 constant, (c) Plot between Drug and sonication time keeping chloroform and X3 constant

6.7.2.1 Morphological study of optimized SLN formulation

Transmission electron microscopy (TEM) image of optimized SLN formulation is shown in Fig. 6.26. The results obtained from drug loaded optimized SLN formulation showed the morphology of the nanoparticles. The smallest vesicle size observed was 30-200 nm at magnification of 290000 X. It could be observed from the TEM image that the formed particles were in nano range, thus, confirming the solid lipid nanoparticle formulation.

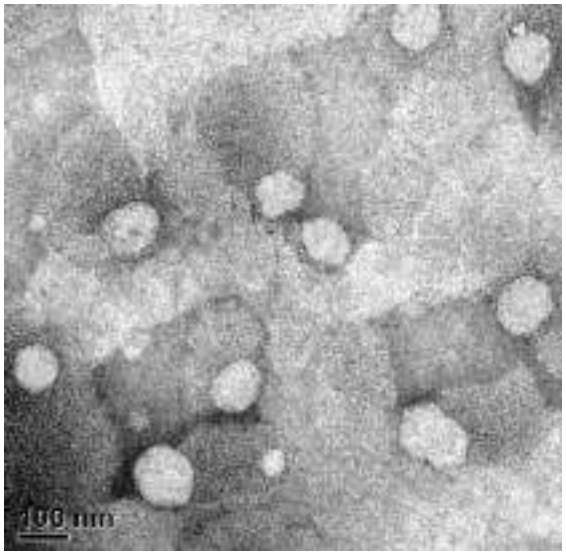


Fig. 6.26 TEM image of optimized SLN formulation (V1) at 290000 X

6.7.2.2 Stability study of optimized SLN dispersion

Table 6.18 and Fig.6.27 represents the stability data of optimized SLN dispersion at 4 ± 3°C and 25 ± 2°C for 15 days. The data showed that there was negligible loss (0.21 %) of entrapped drug at 4 ± 3°C after 15 days of storage. Whereas at 25 ±2°C, comparatively more loss of drug (5.32%) occurred but it was within the limits so the system can be considered as stable. Thus, the results showed that the SLNs are stable at room temperature. As it was observed that the nanoparticles were more stable at refrigerated conditions, so it appears that it is better to provide the same conditions to provide more stability.

Table 6.18

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

Time (in days)	Entrapment efficiency (%) at 4 ± 3°C	Entrapment efficiency (%) at 25 ± 2°C	Encapsulated drug loss (%) at 4 ± 3°C	Encapsulated drug loss (%) at 25 ± 2°C
0	93.7	93.70	0	0
2	93.7	93.56	0	0.15
6	93.7	92.23	0	1.57
10	93.6	90.04	0.11	3.90
15	93.5	88.71	0.21	5.32

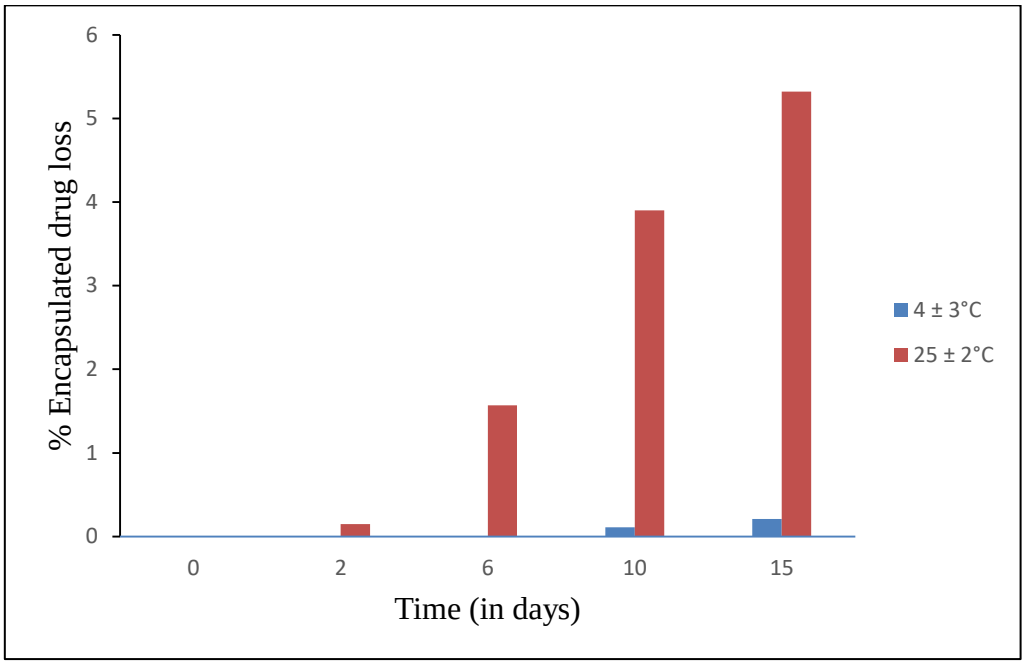


Fig. 6.27 Bar diagram depicting stability study of SLNs at different temperature

6.8 Evaluation of Orlistat SLN capsules

6.8.1 Weight variation of Orlistat SLN capsules

Weight variation of 20 capsules was performed which is shown in table 6.19. The average net weight was found to be 512.7 mg. No capsule was out of limit since all deviations lie within the range of ±7.5%. Thus, the capsules passed the weight variation test.

Table 6.19

Weight variation of Orlistat SLN capsules

S.No.	Weight (mg)	Deviation (%)	Inference
1.	510.2	0.30	Pass
2.	510.4	0.27	Pass
3.	511.3	0.09	Pass
4.	510.6	0.23	Pass
5.	511.8	-0.01	Pass
6.	512.9	-0.22	Pass
7.	516.8	-0.98	Pass
8.	509.6	0.42	Pass
9.	512.3	-0.11	Pass
10.	511.7	0.01	Pass
11.	513.7	-0.38	Pass
12.	516.2	-0.87	Pass
13.	518.5	-1.31	Pass
14.	510.3	0.28	Pass
15.	514.7	-0.57	Pass
16.	511.1	0.13	Pass
17.	513.8	-0.40	Pass
18.	512.5	-0.14	Pass
19.	516.7	-0.96	Pass
20.	508.9	0.56	Pass
	Average= 512.7		

6.8.2 In vitro dissolution study

In vitro dissolution study was performed on USP dissolution apparatus II [Mukund et al, 2016]. The comparative cumulative drug release of both the prepared SLN capsule and the marketed Orlistat capsule is shown in Fig. 6.28. Table 6.20 shows the comparative drug dissolution data of both the prepared SLN capsule and the marketed Orlistat capsule at different time intervals. The drug release of SLN capsule was found to 87.6% while the drug

release of marketed Orlistat capsule was 82.67% in 2 hrs. The drug release of SLN capsule was found to be 1.1 times more than that of the marketed Orlistat capsule.

Table 6.20

Comparative drug dissolution data of prepared SLN capsule and the marketed Orlistat capsule

Time interval (min)	Cumulative % drug release of Orlistat SLN capsule	Cumulative % drug release of Orlistat marketed capsule
5	14.7	11.87
10	24.98	22.84
15	32.71	29.63
20	39.04	33.16
25	47.08	42.23
30	52.25	48.73
45	63.3	61.26
60	77.07	70.42
75	83.25	79.15
90	87.16	81.57
105	87.6	82.03
120	87.6	82.67

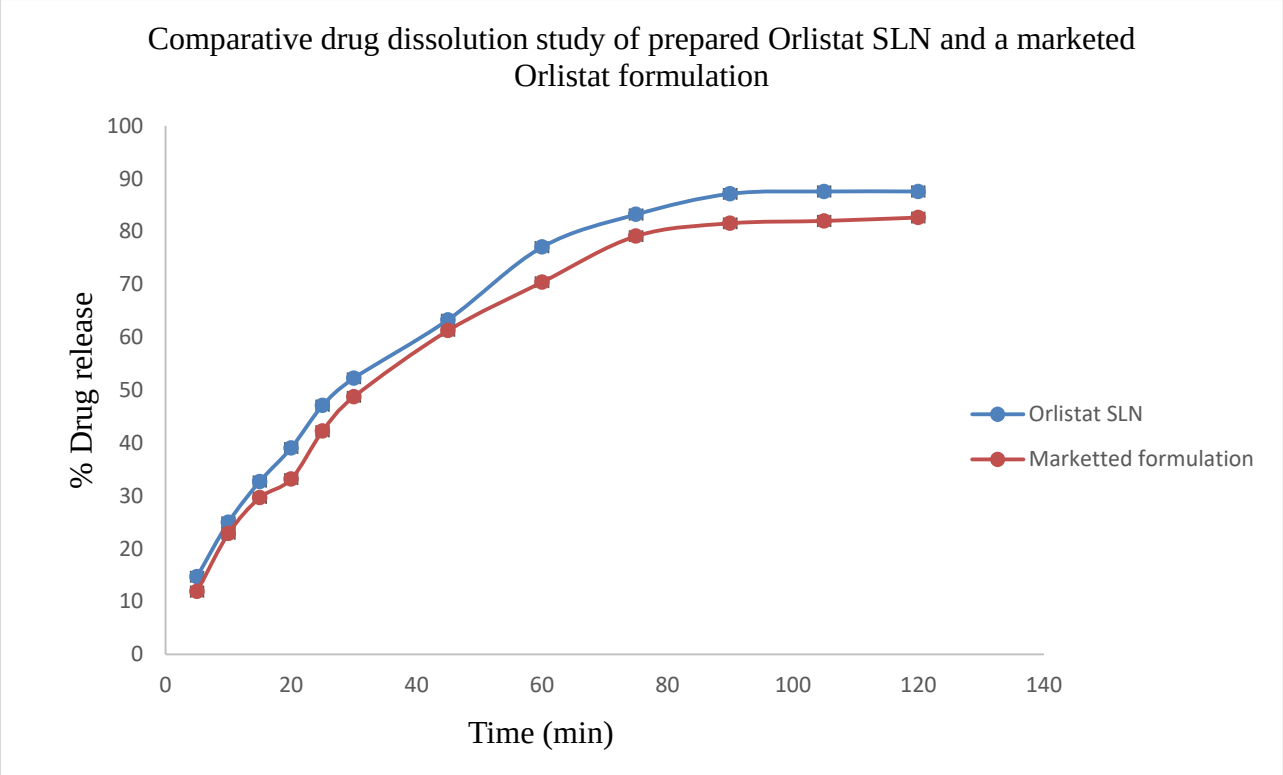


Fig 6.28 Comparative drug dissolution study of prepared Orlistat SLN and a marketed Orlistat formulation

6.8.3 Stability study of prepared SLN capsule

Table 6.21 and Fig.6.29 represents the stability data of SLN capsule at 25 ± 2°C and 50 ± 2°C for 15 days. The data showed that there was negligible decrease (1.25 %) of drug release at 25 ± 2°C after 15 days of storage. Whereas at 50 ± 2°C comparatively more decline of drug release (2.74%) occurred but it was within the limits so the system can be considered as stable. Thus, the results showed that the SLNs capsules are stable at room temperature.

Table 6.21

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

Time (in days)	Drug release (%) at 25 ± 2°C	Drug release (%) at 50 ± 2°C	Decline in drug release (%) at 25 ± 2°C	Decline in drug release (%) at 50 ± 2°C
0	87.6	87.6	0	0
2	87.6	87.2	0	0.45
6	87.4	86.6	0.22	1.14
10	86.8	86.3	0.91	1.48
15	86.5	85.2	1.25	2.74

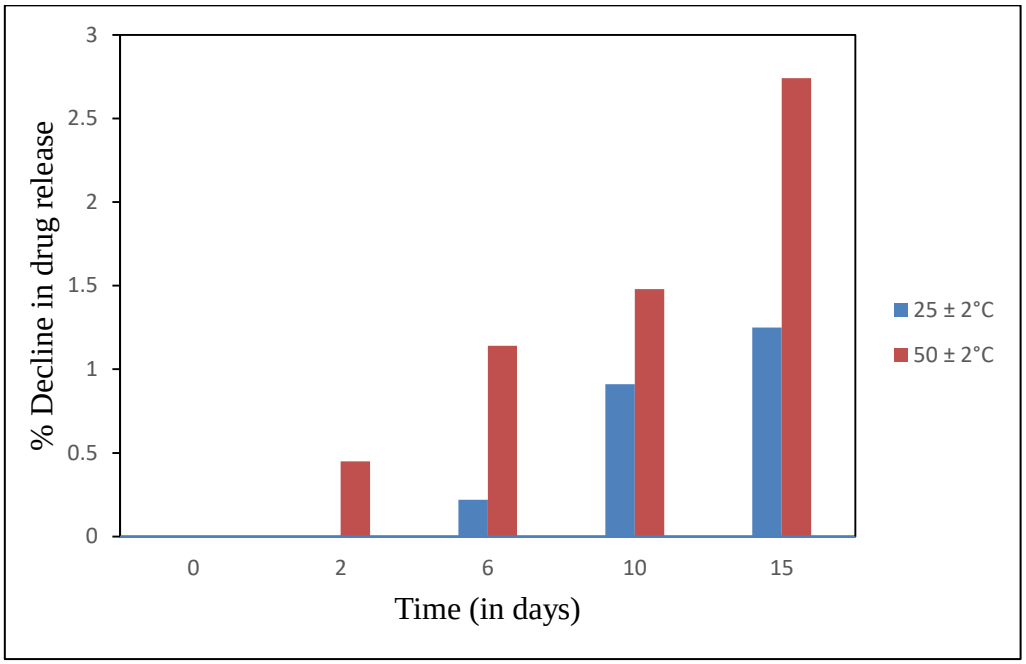


Fig 6.29 Bar diagram depicting stability study of Orlistat SLN capsule at different temperature

6.8.4 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, Peppas and Hixson-Crowell cube root equation. These kinetic models were used to understand the release mechanism of cubosomal formulation. The r^2 and k values of the model equation are shown in table 6.22. The model with r^2 value nearest to 1.000 was considered as the 'best-fit'model for the

formulation. The maximum n values were found to be for kosmeyer peppas model and r^2 values for zero order model, this shows that the release kinetics follows kosmeyer peppas model, the formulation with n > 0.5 indicated release by fickian diffusion. It was found that all the optimized formulation followed fickian, transport as all the values of n were above 0.5. Furthermore, high r^2 values for zero order model, it depicted that the release rate was independent of the concentration of the drug dissolved.

Table 6.22

Various kinetic models of SLNs

Preparation	**Zero Order**	**First Order**	**Higuchi model**	**Hixson Crowell**	**Korsmeyer Peppas**
SLN dispersion	K= 0.849 r^2 = 0.7295	K= 0.016 r^2 = 0.9587	K= 7.695 r^2 = 0.9859	K= 0.005 r^2 = 0.9241	K= 6.075 n = 0.556 r^2 = 0.9928
SLN capsule	K= 0.969 r^2 = 0.4853	K= 0.024 r^2 = 0.9856	K= 8.936 r^2 = 0.9620	K=0.007 r^2 = 0.9426	K= 9.678 n = 0.481 r^2 = 0.9630

CHAPTER 7

SUMMARY AND CONCLUSION

Obesity or being over-weight is a modern day lifestyle disease and is becoming very common in people, affecting all age groups. The obesity management is very important as it may lead to several other severe health problems. Thus, along with healthy, low calorie diet and physical exercise, medication is used for its treatment and management. Since, the medication has to be given through oral route, the poorly soluble drug face many complications in dissolving into the body fluids. So, to enhance the therapeutic absorption and bioavailability of oral dosage form and to improve the efficacy and potency of the dosage form, novel SLN system was developed.

Orlistat was selected as a model drug. The characterization of Orlistat 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 Orlistat in 0.5% w/v iodine solution in DCM was carried to establish a simple and reproducible analytical method for estimation of Orlistat U.V spectrophotometrically. Prescreening studies were performed to decide the range and ratio of drug and organic solvent (chloroform). The results from the prescreening study were implemented in design of expert software by using central composite design. Seventeen formulations F1 – F17 were prepared with varying amount of chloroform, Orlistat, duration of homogenization and duration of sonication. The runs suggested by the software Design Expert® were prepared and were tested for two responses *i.e.* percentage entrapment efficiency and percentage drug release. This data was entered into Design Expert software and 100 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 drug release. The two 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 ± 9%. The optimized SLN formulation was studied for morphology by TEM and SEM which ensured the formation of nanoparticles. The zeta size analysis was carried which presented the vesicle size average range of 212 nm. The drug entrapment efficiency of the SLN dispersion was analyzed. The SLNs were then, filled into capsules. The optimized SLN

capsules was studied for various evaluation parameters such as *in vitro* release, SLN stability and release kinetics. The prepared SLN capsule was compared with the marketed Orlistat capsule preparation to observe the difference in the performance by carrying *in vitro* dissolution study of both the capsules, it was observed that the SLN formulation enhanced the drug release 1.1 times as compared to the marketed Orlistat capsule.

From the different studies which were carried on SLN dispersion and SLN capsule, it could be concluded that the optimized Orlistat SLN capsules presents with a promising formulation to treat obesity 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 SLN capsule of Orlistat to enhance the efficiency of the drug to treat the obesity. 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 involve: *Ex vivo* study of the SLN capsules to treat obesity along with various histopathological studies to ensure the safety profile of the developed system. The SLN system has emerged as a promising approach for the pharmaceuticals. Many researchers have focused their research for developing simple processing techniques to make the production of SLNs more economical. With the advancement and researches carried, SLN system has emerged as a potential delivery system to serve as a suitable delivery system for pharmaceuticals in future.

CHAPTER 8

REFERENCES

Ahlin P., Kristl J., Kobar S., 1998. Optimization of procedure parameters and physical stability of solid lipid nanoparticles in dispersion. Acta Pharm. 48, 257–67.

Aronne LJ., Segal KR., 2003. Weight gain in the treatment of mood disorders. J Clin Psychiatry. 64(Suppl 8),22–29.

Baigent C., Keech A., Kearney PM. 2005. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 366, 1267–1278.

Barbier P, Schneider F., 1987. Syntheses of tetrahydrolipstatin and absolute configuration of tetrahydrolipstatin and lipstatin. Helvetica Chimica Acta. 70 (1), 196–202.

Bummer PM., 2004 .Physical Chemical Considerations of Lipid-Based Oral Drug Delivery—Solid Lipid Nanoparticles. Therapeutic Drug Carrier Systems. Volume 21, Issue 1, 20 pages.

Casadei MA., Cerreto F., Cesa S., Giannuzzo M., Feeney M., Marianecci C., Paolicelli P., 2006. Solid lipid nanoparticles incorporated in dextran hydrogels: A new drug delivery system for oral formulations. International Journal of Pharmaceutics. 325,140–146.

Cavalli R., Gasco MR., Chetoni P., Burgalassi S., 2002. Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. International Journal Pharm. 238, 241 – 245.

Costa P, Lobo JMS., 2001. Modeling and comparison of dissolution profiles. European journal of pharmaceutical sciences. 13, 123-133.

Dash S., Murthy PN., Nath L., Chowdhury P., 2010. Kinetic modeling on drug release from controlled drug delivery systems. Acta Poloniae Pharmaceutica. 67,217-23.

Domb AJ., 1993. Lipospheres for controlled delivery of substances. United States Patent, USS 188837

Ekambaram P., Abdul HA., Priyanka K., 2012 solid lipid nanoparticles: a review. Scientific Reviews Chemical Communications, 2(1), 80-102.

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

Filippatos TD., Derdemezis CS., Elisaf MS., 2009. Effects of Orlistat, Alone or Combined with Hypolipidemic Drugs, on Cardiovascular Risk Factors. Clin Lipidology. 4(3), 331-341.

Flegal KM., Caroll MD., Ogden CL., 2010. Prevalence and trends in obesity among US adults, 1999-2008. JAMA 303(3), 235-241.

Gasco MR., 1993. Method for producing solid lipid microspheres having a narrow size distribution. United states patent, US 188837.

Gohla SH., Dingler A., 2001. Scaling up feasibility of the production of solid lipid nanoparticles (SLN). Pharmazie. 56, 61-63.

Guerciolini R.. 1997. Mode of action of Orlistat. Int J Obes Relat Metab Disord. Suppl 3,S12-23.

Hauptman JB., Jeunet FS., Hartmann D., 1992. Initial studies in humans with the novel gastrointestinal lipase inhibitor Ro180467(tetrahydrolipstatin). Am J Clin Nutr. 55, 309S–13S.

ICH Q1A (R2), 2003. Stability testing of new drug substance and product.

ICH Q2 (R1) 2005. Validation of analytical procedure: Text and methodology.

Jain NK., 1997. Controlled and Novel Drug Delivery. CBS Publishers and Distributors. 1st Edition, 3-28.

Jannin V., Musakhanian J., Marchaud D., 2008. Approach for the development of solid and semi-solid lipid-based formulations. Adv Drug Deliv Rev. 60,734–47.

Jia YF., Yann L., Chia C., Chia HL., Yi HT., 2004. Lipid nano/submicron emulsion as vehicle for topical flurbiprofen delivery. Drug Del. 11, 97-105.

Kalepu S., Manthina M., Padavala V., 2013. Oral lipid-based drug delivery systems – an overview. Acta Pharmaceutica Sinica B. Volume 3, Issue 6, December 2013, Pages 361–372.

Khalil RA., Ahmed A., Kassem MA., Ridi MS., Samra MMA., Awad G., Mansy SS., 2014. Preparation and in vivo Assessment of Nystatin-Loaded Solid Lipid Nanoparticles

for Topical Delivery against Cutaneous Candidiasis. International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering. Vol:8, No:7.

Khare A., Singh I., Pawar P., Grover K., 2016. Design and Evaluation of Voriconazole Loaded Solid Lipid Nanoparticles for Ophthalmic Application. Journal of Drug Delivery. Volume 2016, Article ID 6590361, 11 pages.

Kothamasu SS., Sah A., Asapu US., Arumulla MR., 2009. Orlistat pharmaceutical formulation. WO 2009039157 A2. PCT/US2008/076641. 26 Mar 2009.

Lander R., Manger W., Scouloudis M., Ku A., Davis C., Lee A., 2000. Gaulin homogenization: a mechanistic study. Biotechnol Prog. 16,80–5.

Lau DC., Douketis JD., Morrison KM., Hramiak IM., Sharma AM., Ur E., April 2007. 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children summary. CMAJ. 176 (8), S1–13.

Lin CH., Chen CH., Lin ZC., Fang JY., 2017. Recent advances in oral delivery of drugs and bioactive natural products using solid lipid nanoparticles as the carriers. Journal of Food and Drug Analysis. Volume 25, Issue 2, Pages 219–234.

Lokhandwala H., Deshpande A., Deshpande S., 2013. Kinetic modeling and dissolution profiles comparison: an overview. Internation Journal of Pharma and Biosciences. 4,728-73.

Mukherjee S., Ray S., Thakur RS., 2009. Solid Lipid Nanoparticles: A Modern Formulation Approach in Drug Delivery System. Indian J Pharm Sci. 71(4), 349–358.

Mukund MG., Hurkadale PJ., 2016. Formulation and evaluation of self-emulsifying orlistat tablet to enhance drug release and in vivo performance: factorial design approach. Drug Delivery and Translational Research. Volume 6, Issue 3, pp 276–288.

Obesity and overweight Fact sheet N°311. WHO. January 2015. Retrieved 2 February 2016.

Olbrich C., Gebner A., Kayser O., Muller RH., 2002. Lipid–drug conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazenediaceturate. J Drug Target. 10,387–96.

Omwoyo WN., Ogutu B., Oloo F., Swai H., Kalombo L., Melariri P., Mahanga GM., Gathirwa JW., 2014. Preparation, characterization, and optimization of primaquine-loaded solid lipid nanoparticles. Int J Nanomedicine. 9, 3865–3874.

Padwal R., Li SK., Lau DC., 2004. Long-term pharmacotherapy for obesity and overweight. Cochrane Database Syst Rev (3): CD004094.

Pommier A., Pons M., Kocienski P., 1995. The first total synthesis of (−) lipstatin. Journal of Organic Chemistry. 60 (22), 7334–7339.

Pooja D., Tunki L., Kulhari H., Reddy BB., Sistla R., 2015. Optimization of solid lipid nanoparticles prepared by a single emulsification-solvent evaporation method. Data in Brief. (2),123-126

Pouton CW., 2006. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 29, 278-87.

Rabinarayan P., Padilama S., 2010. Production of Solid Lipid Nanoparticles-Drug Loading and Release Mechanism. Journal of Chemical and Pharmaceutical Research. 2(1),211-227.

Radte M., Souto EB., Muller RH., 2005. Nanostructured Lipid Carriers: a novel generation of solid lipid drug carriers. Pharm Technol Eur. 17(4), 45-50.

Ramteke KH., Dighe PA., Kharat AR., Patil SV., 2014. Mathematical models of drug dissolution: a review. Scholars Academic Journal of Pharmacy. 3,388-396.

Ramteke KH., Joshi SA., Dhole SN., 2012. Solid Lipid Nanoparticle: A Review. IOSR Journal of Pharmacy. Volume 2, Issue 6,PP.34-44

Samyuktha RB., Hari BN., 2011. Niosomal Formulation Of Orlistat: Formulation And In-Vitro Evaluation. Int. J. Drug Dev. & Res. 3(3), 300-311.

Sarmento B., Martins S., Ferreira D., Souto EB., 2007. Oral insulin delivery by means of solid lipid nanoparticles. International Journal of Nanomedicine. 2(4), 743–749.

Sateesha SB., Rao BP., Rajamma AJ., Nargund LVG., 2011. Gastroretentive Orlistat Microspheres: Formulation, Characterization, and In Vitro Evaluation. Dissolution technologies. 73-74.

Schwarz C., Mehnert W., Lucks JS., Müller RH., 1994. Solid lipid nanoparticles (SLN) for controlled drug delivery: I. Production, characterization and sterilization. Journal of Controlled Release, 30, 83-96.

Shah C., Shah V., Upadhyay U., 2011. Solid lipid nanoparticles: a review. Current pharma research. 1(4), 351-368.

Shick SM., Wing RR., Klem ML., McGuire MT., Hill JO., Seagle H., April 1998. Persons successful at long-term weight loss and maintenance continue to consume a low-energy, low-fat diet. J Am Diet Assoc. 98(4), 408–13.

Shidhaye SS., Vaidya R., Sutar S., Patwardhan A., Kadam VJ., 2008. Solid lipid nanoparticles and nanostructured lipid carriers-innovative generations of solid lipid carriers. Curr Drug Deliv. 5(4), 324-31.

Siekmann B., Westesen K., 1996. Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions. European J. Pharm. Biopharm, 43,104-109.

Siepmann J., Peppas NA., 2012. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Advanced drug delivery reviews. 64,163-174.

Singhavi G., 2011. Review: In vitro drug release characterization models. International journal of Pharmaceutical studies and research. 2,77-84.

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

Teja LG., 2015. Investigation on solid lipid nanoparticles formulations. J. Pharm. Sci. & Res. Vol. 7(3), 155-158

The Indian Pharmacopoeia commission., 2014 . Indian pharmacopoeia, vol.1. pp 153.

Torgerson J., Hauptman J., Boldrin M., Sjöström L., 2004. Xenical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of Orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care. 27 (1), 155–61.

Ugazio E., Cavalli R., Gasco MR., 2002. Incorporation of cyclosporin A in solid lipid nanoparticles (SLN). International Journal of Pharmaceutics. 241, 341–344.

USP 30/NF 25., 2007. The United States Pharmacopeia 30/National Formulary 25. United States Pharmacopeial Convention, Inc., USA.

Venkateswarlu K., Naik SBT., Chandrasekhar KB., 2016. Formulation and in-vitro evaluation of orlistat orodispersible tablets for enhancement of dissolution rate. Int J Pharm Pharm Sci. Vol 8, Issue 4,236-241.

Venkateswarlu V., Manjunath K., 2004. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. 2004. Journal of Controlled Release, 95, 627-638.

Vuddisa SK., Subramanian S., Raavi S., 2014. Preparation and Characterization of Candesartan Cilexetil Solid Lipid Nanoparticulate Capsules. International Journal of Pharma Research & Review. 3(12),26-31

Yang S., Zhu J., Lu Y., Liang B., Yang C. 1999. Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharmaceutical Research, 16(5), 1-7.

APPENDIX

CERTIFICATE OF ANALYSIS OF DRUG (COA)

BILLS BIOTECH PVT. LTD.
Plot No.-P/01, Biotech Park Phase-I, GIDC, Manjusar Industrial Estate
Village-Manjusar, Savli-Vadodara-391775

Format No: BBPL/QC/S/003/F/03-00

CERTIFICATE OF ANALYSIS

Product Name : ORLISTAT					
Batch No.	:	ORL160917	**A.R.NO.**	:	BFP/160013
Mfg.date	:	09/2016	**Batch size**	:	11.7 KG
Re-test date	:	09/2019			
Storage condition	:	**Preserved in well-closed containers between 2°c to 8°c.**			

TESTS	SPECIFICATION	RESULTS
Description	White to off white powder.	White powder
Solubility	It is freely soluble in chloroform, Insoluble in water	Complies
Identification		
A. Test A (By IR)	The IR absorption spectrum of the sample concordant to that of the spectrum of orlistat standard.	Complies
B. Test B (By HPLC)	The retention time of the major peak of the sample solution corresponds to that of the *standard solution, in the Assay.*	Complies
Melting Point	Between 40°C and 46°C	42.4°C
Water content by KF	Not more than 0.8 %	0.0 %
Residue on ignition	Not more than 0.2%	0.07 %
Heavy metals (As per Method II)	Not more than 0.002% (20 ppm)	Less than 20ppm
Specific optical rotation	Between -43.0° and -51.0° at 20°C	- 49.9°
Orlistat Related compounds- organic impurities:		
Related Substances- By HPLC	Any Single Impurity : NMT 1.0 %	Complies
	Total impurities: NMT 3.0 %	1.81%
ASSAY by HPLC *(on anhydrous basis)*	95.0% to 101.5% w/w	97.68 %
Residual solvents by GC	Hexane : NMT 290 ppm	50.2 ppm
	Methanol : NMT 3000 ppm	Not detected
	Heptane : NMT 5000 ppm	Not detected

Opinion: The product complies / ~~does not comply~~ above tests as per In house specifications.

Checked by :	**Approved by :**
Date: 03/10/2016	Date: 03/10/2016

Page 1 of 1