"FORMULATION AND OPTIMIZATION OF GAMMA ORYZANOL NIOSOMAL GEL FOR SKIN CANCER USING QbD APPROACH"

A THESIS SUBMITTED TO

NIRMA UNIVERSITY

IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF

MASTER OF PHARMACY

IN

PHARMACEUTICAL TECHNOLOGY & BIOPHARMACEUTICS

BY

ANKIT GOTECHA (13MPH101), B. PHARM.

UNDER THE GUIDANCE OF

Dr. SHITAL BUTANI – GUIDE
ASSOCIATE PROFESSOR, DEPARTMENT OF PHARMACEUTICS

Dr. SHITAL PANCHAL – JOINT GUIDE
ASSISTANT PROFESSOR, DEPARTMENT OF PHARMACOLOGY

Department of Pharmaceutics
Institute of Pharmacy
Nirma University
Ahmedabad-382481
Gujarat, India.

MAY 2015

CERTIFICATE

This is to certify that the dissertation work entitled "Formulation and Optimization of Gamma Oryzanol Niosomal Gel for Skin Cancer Using QbD Approach" submitted by Mr. Ankit Gotecha with Regn. No. (13MPH101) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Technology and Biopharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

Guide:

Dr. SHITAL BUTANI
M. Pharm., Ph.D.
Assistant Professor,
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University

Co-Guide:

Dr. SHITAL PANCHAL
M. Pharm., Ph.D.,
Assistant Professor,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

Forwarded Through:

PROF. TEJAL MEHTA
M. Pharm., Ph.D.,
Professor and Head,
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University

PROF. MANJUNATH GHATE
M. Pharm., Ph.D.,
Director,
Institute of Pharmacy,
Nirma University

Date: 24th May, 2015

DECLARATION

I hereby declare that the dissertation entitled "Formulation and Optimization of Gamma Oryzanol Niosomal Gel for Skin Cancer using QbD Approach" is based on the original work carried out by me under the guidance of Dr. Shital Butani, Assistant professor, Department of Pharmaceutics and joint-guidance of Dr Shital Panchal, Assistant Professor, Department of Pharmacology, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

Mr. Ankit Gotecha (13mph101)
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University,
Sarkhej - Gandhinagar Highway,
Ahmedabad-382481,
Gujarat, India

Date: 24th may, 2015

Acknowledgement

Almighty is the supreme power with whose blessings this work has been possible. I believe in god, the maker of this beautiful creation. I surrender my heart to him and thank him with the bottom of my heart for making my work and standing as a shadow to help me out in every difficult situation of mine. The tour of this work began with faith and blessings of God. Thereby, it is a matter of gratification, joy, and delightment to fetch my gratitude to each and everyone who had made this journey worthwhile.

To work on this dissertation is not only fulfilling my dream but also the dreams of my family who have taken lots of pain in the completion of my higher studies. I am very grateful to My Parents **Mr. Kamlesh Gotecha & Mrs. Jagruti Gotecha** for taking care of me, helping me in each and every way and for moulding me to make my carrier and aiding to make my dreams come true. I am thankful to My Brother **Vishal** for his love and support.

I am thankful to **Dr. Manjunath D. Ghate**, Director of Institute of pharmacy, Nirma University, Ahmedabad for his knowledgeable guidance and continuous support for the whole year. And giving us all possible helps as well as facility for work.

I consider myself most lucky to work under the guidance of **Dr. Shital Butani**, Associate Professor, Department of Pharamceutics, Institute of Pharmacy, Nirma University, Ahmedabad for her guidance, support, warmth and constant encouragement she had provided throughout the time. I have been extremely lucky for having a friendly supervisor without her positive attitude towards my project have been crucial for this thesis.

With profound appreciation, I express my warm gratitude to my other guide Dr. Shital Panchal for giving her useful and valuable time and guidance at every level of this project.

I am deeply indebted and thankful to **Dr. Tejal Mehta,** head of department of pharmaceutics and all the faculty members **Dr. Jigar Shah, Dr. Mayur M. Patel and**

Dr. Renuka Mishra for their keen interest and valuable support. They were always available to give me the suggestions course of this work.

I would like to acknowledge all the HODs of each department for their constant support and help whenever required during the project.

"Friends are like pearls of deep sea – Very rare and very precious"

A special word of gratitude to my friends **Pankti, Dhara, and Mimansa,** who were always there besides me with the hand of support and encouragement to make this effort a successful task. They have always helped me to achieve my goals and made my tough moments lighter with fun and laughter.

I am also thankful to my batch mates Anuradha, Bhranti, Bhavna, Darshan, Jitendra, khushali, khushboo, kripali, kasim, Zahid, Monil, Ronak, Pooja, Parth Dave, Parth Patel, Shreyash. A special word of gratitude to **Sumit, Meet, Abhishek, Jinal, Mansi, Gayatri, Payal** and **Twisha** without whose support this task could not be accomplished.

I am also grateful and my heartly thanks to PhD scholar **Nimit Chokasi, Neha Shah, Manali, Abhishek Jha and Neeraj sir**

I also wish to acknowledge **Shailesh bhai, Mukesh bhai, Shilpa mam** and **Rohit bhai** for providing me all the materials required in my work. I also take this opportunity to convey my sincere thanks to teaching and non-teaching staff of Institute of pharmacy Nirma University, Ahmedabad especially, Librarians for their kind cooperation throughout.

Finally, even words are not enough to express my gratitude to all those who have been important for this successful work. I take these all memories candidly as I step to the new stepping stones of my life.

Thanking you once again…I wish everyone best of luck for their future.

Ankit Gotecha

INDEX

List of Tables

List of Figures/Flowchart

LIST OF ABBREVIATIONS

OZ	**:** Gamma oryzanol
KBr	**:** Potassium bromide
PBS	**:** Phosphate buffer saline
mg	**:** Milligram
µg	**:** Microgram
FTIR	**:** Fourier transform Infra-Red
UV	**:** Ultra violet
&	**:** And
^{o}C	**:** Degree centigrade
RH	**:** Relative humidity
W/W	**:** Weight/Weight
TEM	**:** Transmission electron microscope
pH	**:** Potency of hydrogen ion
EE	**:** Entrapment efficiency
%	**:** Percentage
SPE	**:** Solid phase extraction
cP	**:** centi Poise
ml	**:** millilitre
ml/min	**:** millilitre/minute
rpm	**:** rotation per minute
G	**:** Gauge
DCP	**:** Dicetly phosphate
PDI	**:** Polydispersity index
mV	**:** millivolt
Conc.	**:** Concentration
Sr no.	**:**Serial number
CQAs	**:** Critical quality attributes
CMAs	**:** Critical material attributes
CPPs	**:** Critical process parameters
Temp	**:** Temperature

ABSTRACT

"Formulation and optimization of gamma oryzanol niosomal gel for skin cancer using QbD approach"

The present research work is aimed at the development and optimization of gamma oryzanol (OZ) niosomes, to formulate an aqueous gel containing niosomes. OZ niosomes were prepared by two methods, Solvent injection method and thin film hydration method by using span 60 as a non ionic surfactant in both processes. Effect of various parameters such as Temperature, injection flow rate, span cholesterol ratio, span drug ratio, stirring speed, sonication, concentration of dicetyl phosphate (DCP) as a negative charge inducer, needle size and volume of hydration have been taken into consideration while preparing the niosomes. Full factorial design (2^3) was applied for the optimization of final formulation by taking drug: span ratio (A), volume of hydration (B) and stirring speed (C) as independent variables. Optimized batch was evaluated for average vesicle size, entrapment efficiency, zeta potential and TEM analysis and then incorporated in to carbopol gel. The gel was evaluated for viscosity, pH, appearance, spreadability, % drug diffusion and % drug retention of niosomal formulation. Draize's skin irritation study of niosomal gel was performed on skin of conscious rabbits and then kept for stability studies. FTIR spectra indicated that drug was pure and compatible with all selected excipients. Solvent injection method was selected over thin film hydration method for further optimization because of its short process duration, easy recovery, uni-lamellar vesicles, better entrapment efficiency and desired vesicle size. From factorial batches, batch having composition of factor A (1:5), B (75) and C (2500) showed 110.49 nm average vesicle size, 78.31 % drug entrapment efficiency was selected as optimized batch. The scale up of optimized batch also showed similar results. Hence optimized batch was formulated in to gel which showed 70.1 % drug retention. Developed niosomal gel showed poor diffusion and higher drug retention when compared with conventional gel. The skin irritation study showed no positive effect of redness or edema on rabbit skin. The optimized niosomal gel showed good storage stability up to 1 month at room temperature.

CHAPTER 1

AIM AND OBJECTIVE

1. AIM & OBJECTIVES

- Topical drug delivery system is having various advantages over conventional dosage form like avoiding first pass metabolism, delivering drug without ingestion in to the GIT, local delivery of drug at the site of application and patient compliance. Though it is having advantages over conventional dosage form, stratum cornium is a major barrier for skin permeability of drug. To overcome such drawbacks, novel nano formulations like niosomes are prepared.
- Niosomes because of its small size range i.e. between 10-100 nm and vesicular elasticity possesses various unique properties which plays key role in increasing the range of formulation for transdermal delivery in terms of improving drug solubility, permeability, stability and skin retention. These properties can be used to overcome some of the drawbacks found with transdermal drug delivery system.
- Gamma oryzanol have been proved for its various activities like anticancer, antioxidant, etc. Also it is biodegradable and nontoxic but because of its problems of poor solubility, and low bioavailability, it is found to be a promising drug to explore further by forming niosomal gel for better and therapeutic efficacy and controlled drug release.

1.1 Aim of research work

- The Aim of the present research work was to formulate and optimize gamma Oryzanol niosomes and to incorporate it in a gel for skin cancer.

1.2 Objectives

- To formulate gamma Oryzanol niosomes, a lipophilic drug by usingsuitable technique.
- To optimize niosomal formulation using DOE (Design of Experiment).
- To incorporate developed niosomes in to gel and compare it with conventional gel with respect to improvement in drug retention in skin.

CHAPTER 2

INTRODUCTION

2. INTRODUCTION

2.1 Introduction to transdermal drug delivery system [1–3]

Most drugs are either absorbed into the body through the digestive system or injected into subcutaneous tissue or muscle. An alternative route, transdermal (transcutaneous) drug administration, enables a drug enclosed within an adhesive skin patch to pass across the epidermis and into the blood vessels of the dermis. It can also be described as the passage of substance from the outside of the skin through its various layers into the bloodstream. The drug is released continuously at a controlled rate over interlude. This method of administration is especially useful for drugs that are quickly eliminated from the body because such drugs, if taken in other forms, would have to be taken quite frequently. Because the major barrier to penetration of most drugs is the stratum corneum, transdermal absorption is most rapid in regions of the skin where this layer is thin, such as the scrotum, face, and scalp. Figure 2.1 shows sectional view of skin.

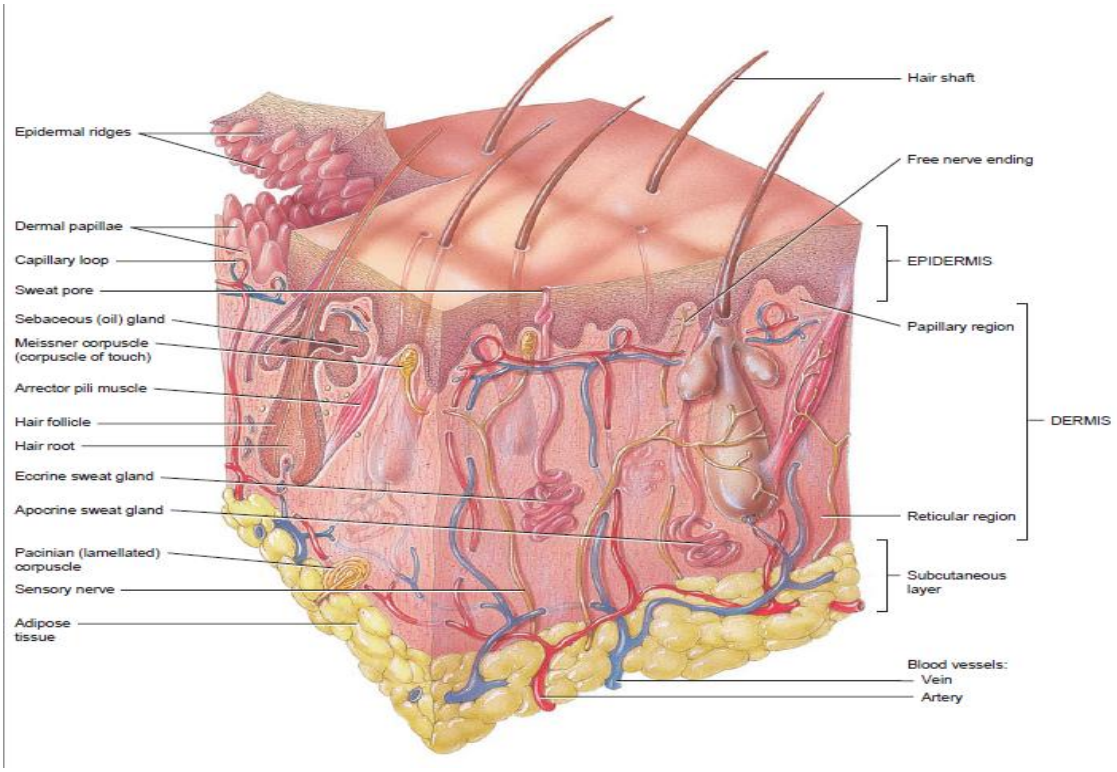


Figure 2.1 Sectional view of skin

Advantages of transdermal delivery system [2,3]

- The system avoids the chemically hostile GI environment
- No GI distress or other physiological contraindications of the oral route
- Can provide adequate absorption of certain drugs
- Increased patient compliance
- Avoids first-pass effect
- Allows effective use of drugs with short biological half-life
- Allow administration of drugs with narrow therapeutic windows
- Provides controlled plasma levels of very potent drugs
- Drug input can be promptly interrupted when toxicity occurs

Disadvantages of TDS [2,3]

- Drug that require high blood levels cannot be administered
- Adhesive may not adhere well to all types of skin
- Drug or drug formulation may cause skin irritation or sensitization
- Uncomfortable to wear
- May not be economical

Factors influencing the permeation of drugs [3,4,7]

- Skin structure and its properties
- The penetrating molecule and its physical-chemical relationship to skin and the delivery platform
- The platform or delivery system carrying the penetrant
- The combination of skin, penetrant, and delivery system

Factor influence the transdermal route [4]

- Physicochemical properties of penetrant (pKa, molecular size, stability, binding affinity, solubility, partition coefficient)
- Integrity and thickness of stratum corneum
- Density of sweat glands and follicles
- Skin hydration

- Metabolism
- Vehicle effects

Fundamentals of Skin Permeation:- Stratum Corneum as the Skin permeation Barrier: [1,2,7]

- Skin while being an effective barrier against environmental assaults, is not completely impermeable.
- Certain drugs are either inherently permeable, or can be formulated to penetrate the skin.
- Outer most layer of skin (the stratum corneum) is made up of dead epidermal cells (keratinocytes) and is the primary barrier to drug delivery.
- Once this layer is penetrated, the feasibility of drug delivery is greatly improved.
- Three distinct layers: the stratum corneum (15μm thick), the viable dermis (150 μm thick), and the papillary layer of the dermis (100-200 μm thickness).
- The papillary arterial plexus forms pierced in various places by two types of potential diffusion shunts; hair follicles and sweat glands.
- The foreign agents may be able to penetrate into the skin via these skin appendages at a rate faster than that through the intact area of the stratum corneum.
- The data accumulated to date have demonstrated that skin permeation is primarily controlled by diffusion across the bulk of stratum corneum.

Fick's First Law of Diffusion: Percutaneous absorption of most drugs is a passive-diffusion process that can be described by Fick's first law of diffusion[5]

$$\mathbf{dM/dt = JT = AP*\Delta C}$$

Where,

JT = Total flux transported through a unit area of skin per unit time in steady state (mcg/hr)

A = Area of the skin

P = Effective permeability coefficient

ΔC = Drug concentration gradient across the skin

Examples of Transdermal Applications [4,5,6]

Monolithic systems

- Nitrodur and Nitrodisc
- Manufacture drug reservoir with polymer with subsequent casting and drying
- Punch from sheet or sliced cylinder
- Assembled with the system backing, peripheral adhesive and protective liner

Membrane-controlled transdermal system

- Transderm-Nitro, Transderm-Scop
- Form-fill-seal from lamination process

2.2 Introduction to Novel drug delivery system (NDDS) [8, 9]

In order for advancements on the global level various new drug delivery technologies are adopted in the drug discovery, development and R&D focused pharma industries. NDDS gives great number of opportunities to the formulation scientists to overcome various challenges posed by conventional drug delivery, thus helps in improvement of patients with particular acute and chronic diseases. The method of delivery of drug has considerable effect on its efficacy. Maximum drugs having a particular concentration range within which they shows required therapeutic effect & going above or below that concentration range either becomes toxic or produce no therapeutic benefit at all. New ideas on controlling the various ADME factors, pharmacodynamics, nonspecific toxicity, and efficacy of drugs were established. These all new approaches as a whole termed as novel drug delivery systems (NDDS) which mainly focus attention on the minimization of drug degradation and loss, prevention of harmful side effects and to increase bioavailability of drugs and fraction of drug accumulated in the required zone. Various drug delivery systems are presently under developing stage which includes various drug carriers such as microcapsules, lipoproteins, micelles, liposomes, polymeric nanoparticle, niosomesand dendrimers. Among all targeting is the most important property of any carrier because of its ability to direct the drug loaded system to the site of interest which involves two mechanisms basically:

i. Passive targeting (involves accumulation of therapeutic agent at the site of action)
ii. Active targeting (involves surface functionalization of drug carriers with ligands that are selectively identified by receptors on the cells of interest)

Potential release mechanisms involve:

(i) Desorption of surface-bound /adsorbed drugs;

(ii) Diffusion through the carrier matrix;

(iii) Diffusion (in the case of nanocapsules) through the carrier wall;

(iv) Carrier matrix erosion and

(v) Combined erosion /diffusion process.

Novel drug delivery systems (NDDS) have various advantages over conventional drug therapy, some of which includes better therapy by increasing the efficiency and duration

of drug activity, improved patient compliance by decreasing the dosing frequency and suitable routes of administration. Also site specific delivery can be done to reduce the unwanted adverse effects. Common advantages of NDDS are as mentioned below:

2.2.1 Advantages of novel drug delivery systems [8]

- Solubility enhancement
- Improved bioavailability
- Great protection from toxicity offered by fluctuations in the dosage forms
- Enhancement of in vivo activity or pharmacodynamics
- Better stability of dosage forms
- Improved tissue macrophages distribution
- Sustained delivery of conventional drug can be achieved
- Protection from physical and chemical degradation inside GIT tract
- Targeting of drug substances at a particular site inside the body.

2.2.2 Colloidal drug delivery carriers [10]

Carrier mediated drug delivery appeared as one of the most promising technique by which treatment of various diseases can be done in a more efficient way. The main reason of its popularity being counted is because of its better stability profiles, improved loading capabilities and control on various physicochemical properties. These systems are being formulated with the objective of changing the distribution profile of drug inside the body and thus help in improving its therapeutic efficacy by decreasing the toxicity. Colloidal drug carrier systems e.g. Nanoparticle dispersions, nanocrystals, vesicular systems like liposomes, Niosomes, sphingosomes, niosomes, transfersomes, aquasomes, ufasomes, and so forth which consists of very small particles in size range of 10-100 nm diameter has proved to have great potential in novel drug delivery systems. While formulating systems containing these novel carriers, the main aim is to achieve formulation system with optimized drug loading and release profiles, long shelf life and less toxicity. The incorporated loaded drug forms the structure of the system and may even affect it due to interactions at the molecular level, especially in case of drug having amphiphilic or lipophilic nature.

2.3 Introduction to Vesicular drug delivery system [11,12]

Vesicular drug delivery system can be defined as highly ordered assemblies consisting of one or more concentric bilayers formed as a result of self-assembling of amphiphilic building blocks in presence of water. The biologic origin of lipid based vesicles was first reported by Bingham in 1965 and hence was named as Bingham Bodies. Vesicular drug delivery systems are particularly important for targeted delivery of drugs because of their ability to localize the activity of drug at the site or organ of action thereby lowering its concentration at the other sites in body.

2.3.1 Advantages of Vesicular Drug Delivery System [12, 13]

- Both hydrophilic and hydrophobic drugs can be easily encapsulated.
- Bioavailability of drugs can also be improved.
- Elimination of rapidly metabolizable drug can be delayed.
- Circulation life time of drugs in the body can be prolonged.
- Targeted delivery of drugs can often be achieved.
- Stability issues of liable drugs can be resolved.
- Toxicity issues of certain drugs can often be resolved.

2.4 Introduction to Niosome [12-15]

Niosome is a non-ionic surfactant-based liposome. They are formed by the self-assembly of non-ionic surfactant with or without cholesterol in aqueous media resulting in closed bilayer structure. The assembly into closed bilayers is rarely spontaneous and usually involves some input of energy such as physical agitation or heat. They are structurally similar to liposomes in having a bilayer, however, the materials used to prepare niosomes make them more stable and thus niosomes offer many more advantages over liposomes. The sizes of niosomes lie in submicron range mainly in between 10 nm to 100 nm. The first niosome formulations were developed and patented by L'Oreal in 1975.

Niosomes are capable of encapsulating both hydrophilic and lipophilic substances where the former usually are either entrapped in vesicular aqueous core or adsorbed on the bilayer surfaces while the latter are encapsulated within the bilayer. Hence the hydrophobic parts of the molecule are shielded from the aqueous solvent and the hydrophilic head groups enjoy maximum contact with same. Cosmetic industry was the place for the first account of niosome production after which a large number of niosome applications in drug delivery have been explored. Non-ionic surfactants have more chemical stability against both oxidation and temperature in comparison to phospholipids, the main constituent of liposomes, thus requires less care in handling and storage. Furthermore, greater versatility and lower cost make this type of vesicles more attractive in drug, gene and vaccine delivery. From the pharmaceutical manufacturing stand of view, the superiority of niosomes is the ease of their production in large scale without the use of pharmaceutically unacceptable solvents. Although the niosome has better chemical stability in storage but the physical instability during dispersion may be equivalent to that of the liposome. During dispersion, both liposomes and niosomes are at risk of aggregation, fusion, leakage of drugs, or hydrolysis of encapsulated drugs. The figure 2.2 shows structure of niosome.

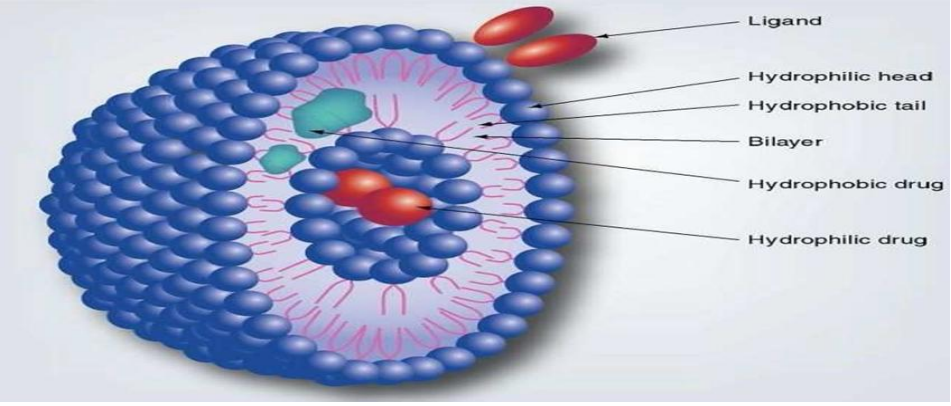


Figure 2.2 Structure of Niosome

2.4.1 Chemical Composition of Niosome [11,13-16]

1. Cholesterol

The most common additive found in niosomal systems is cholesterol which is known to abolish the gel to liquid phase transition of niosomal systems, resulting in less leakiness of the vesicles and improved niosomes stability. Cholesterol is used to complete the hydrophobic moiety of high HLB single alkyl chain non-ionic surfactants for vesicle formation. In general, it has been found that a molar ratio of 1:1 between cholesterol and non-ionic surfactants is an optimal ratio for the formulation of physically stable niosomal vesicles. The minimum amount of CHOL required to form vesicles without evoking surfactant aggregates or other irregular structures depended on the type of surfactant and it's HLB.

2. Nonionic surfactants

The surfactants play a major role in the formation of niosomes. Formation of niosomes requires an amphiphilic molecule composed of two main parts, a polar or hydrophilic head group and a non-polar or hydrophobic tail.The lipophilic moiety of amphiphile molecule may contain one, two or three alkyl or perfluoroalkyl groups or in some cases, a single steroidal group Alkyl ethers, alkyl esters, alkyl amides, fatty acids and amino acids are the main non-ionic surfactant classes used for niosome production. However, the most frequently used surfactants in niosomes formulations are sorbitan monoesters (Spans®, Figure 2.3). The versatility of compounds capable of forming vesicle is due to the

presence of different and various polar head groups attached to saturated or unsaturated alkyl chain(s) composed of 12 to 18 carbon atoms (C_{12}-C_{18}).

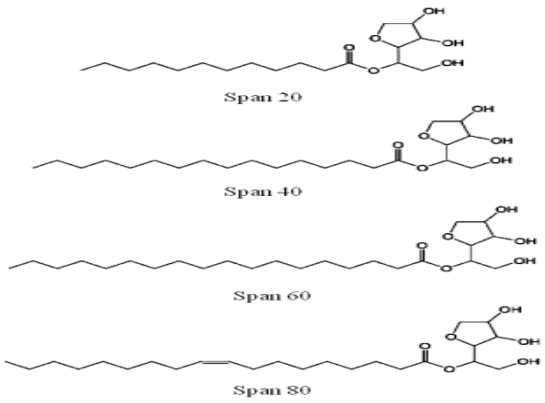


Figure 2.3 Chemical structure of most frequently used surfactants in niosomes formulations, sorbitan monoesters (Spans®)

3. Charged molecules

Charged molecules may be incorporated into vesicular formulation to enhance the electrostatic stability of vesicle, to increase the encapsulation or adsorption of charged molecules, and to orient the vesicles for better specific interaction with target cells. Dicetyl phosphate (DCP) is the most used negative charged molecule in bilayers

2.4.2 Factors affecting the formation of niosomes [14–17]

1. Type of surfactants

Type of the surfactants influences encapsulation efficiency, toxicity, and stability of niosomes. The first niosomes were formulated using cholesterol and single-chain surfactants such as alkyl oxyethylenes. The alkyl group chain length is usually from C12–C18. The hydrophilic- lipophilic balance (HLB) is a good indicator of the vesicle forming ability of any surfactant. Uchegbu et al (1995, 1998) reported that the sorbitanmonostearate (Span) surfactants with HLB values between 4 and 8 were found to be compatible with vesicle formation. As the HLB value increases above 10, it is necessary to increase the cholesterol concentration in order to compensate the effect of the larger head groups on the critical packing parameter (CPP). Polyglycerolmonoalkyl

ethers and polyoxylate analogues are the most widely used single-chain surfactants. However, it must be noted that they possess less encapsulation efficiency in the presence of cholesterol. Etheric surfactants have also been used to form niosomes. These types of surfactants are composed of single-chain, monoalkyl or dialkyl chain. The latest ones are similar to phospholipids and possess higher encapsulation efficiency. Ester type amphyphilic surfactants are also used for niosome formulation. They are degraded by esterases, triglycerides and fatty acids. Although these types of surfactants are less stable than ether type ones, they possess less toxicity. Furthermore, glucosides of myristil, cethyl and stearyl alcohols also form niosomes.

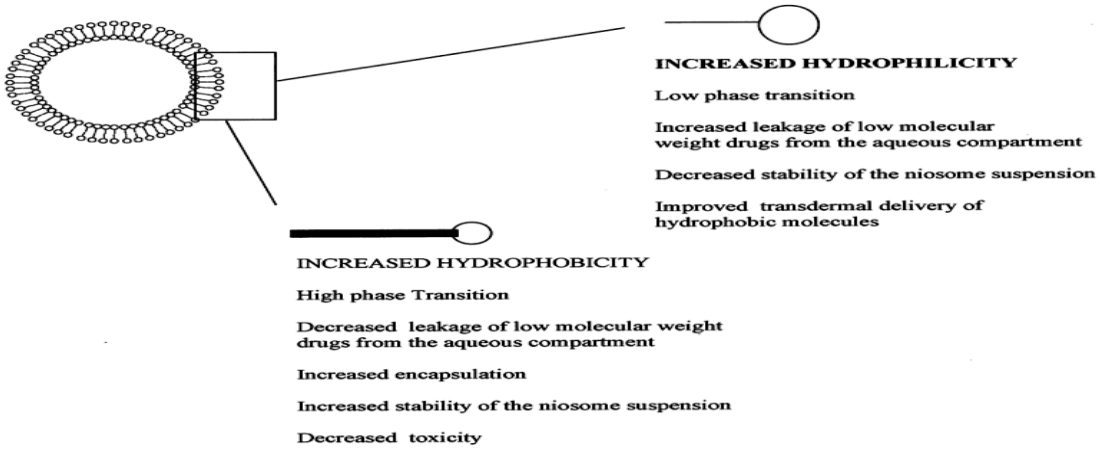


Figure 2.4 Effect of the choice of niosome forming surfactant on the properties of the niosome dispersion.

The shapes of the spontaneously formed association colloids can be predicted with considerable conviction using nominal geometric parameters of the surfactant molecule. The critical packing parameter (CPP) was discussed by Israelachvili et al.and defined by relation:

$$\text{CPP} = V \div lc \times a0$$

Where,

V = Tail volume of the molecule,

a0 = Surface area of hydrophilic head group,

lc = Length of hydrocarbon chain.

According to the CPP value, the shape and size of the equilibrium aggregate should evolve from spherical micelles (CPP ≤ 1/3) to cylindrical micelles (1/3 ≤ CPP ≤ 1/2), bilayers (1/2 ≤ CPP ≤ 1) or inverse micelles (CPP > 1), as shown in figure 2.5.

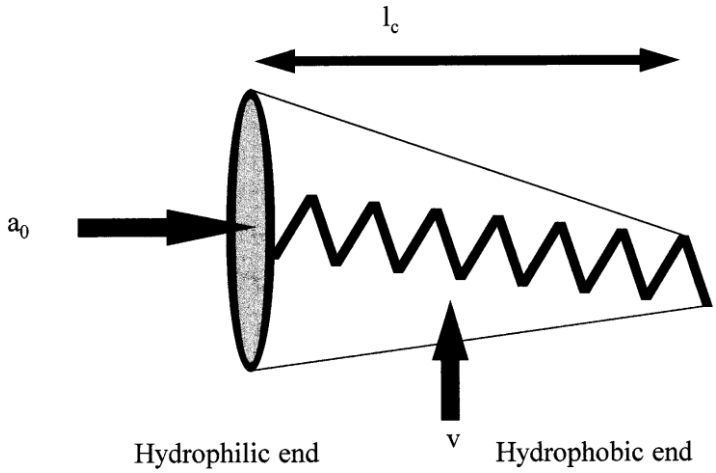


Figure 2.5 Schematic representation of a surfactant

2. Surfactant/Lipid and Surfactant/Water Ratios

Other important parameters are the level of surfactant/lipid and the surfactant/water ratio. The surfactant/lipid ratio is generally 10–30 mM (1–2.5% w/w). If the level of surfactant/lipid is too high, increasing the surfactant/lipid level increases the total amount of drug encapsulated. Change in the surfactant/water ratio during the hydration process may affect the system's microstructure and thus, the system's properties.

3. Cholesterol

Steroids are important components of cell membranes and their presence in membranes brings about significant changes with regard to bilayer stability, fluidity and permeability. Cholesterol, a natural steroid, is the most commonly used membrane additive and can be incorporated to bilayers at high molar ratios. Cholesterol by itself, however, does not form bilayer vesicles. It is usually included in a 1:1 molar ratio in most formulations to prevent vesicle aggregation by the inclusion of molecules that stabilize the system against the formation of aggregates by repulsive steric or electrostatic effects. It leads to the transition from the gel state to liquid phase in niosome systems. As a result, niosomes

become less leaky. An increase in cholesterol content of the bilayers may decrease the release rate of encapsulated material and therefore an increase of the rigidity of the bilayer is obtained.

4. Charge inducer

As is the case with liposomes, charged phospholipids such as dicethyl phosphate (DCP) and stearyl amine (SA) have been used to produce charge in niosome formulations. The former molecule provides negative charge to vesicles whereas the later one is used in the preparation of positively charged (cationic) niosomes. The charge on the niosomes provides physical stability during storage of niosomal formulation due to repulsive force.

5. Nature of the Drug

One of the overlooked factors is the influence of the nature of the encapsulated drug on vesicle formation. Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size. The hydrophilic or lipophilic nature of the drug affects degree of entrapment. In choosing a suitable drug tobe delivered by niosomes, it should be borne in mind that niosomes encapsulating hydrophobic drugs and macromolecules are more stable than niosomes encapsulating low molecular weight drugs. These factors will also affect niosome stability in vivo. In addition transdermal drug delivery appears possible with hydrophobic or amphiphilic molecules. Figure 2.6 and table 2.1 shows effect of drug on niosomal properties.

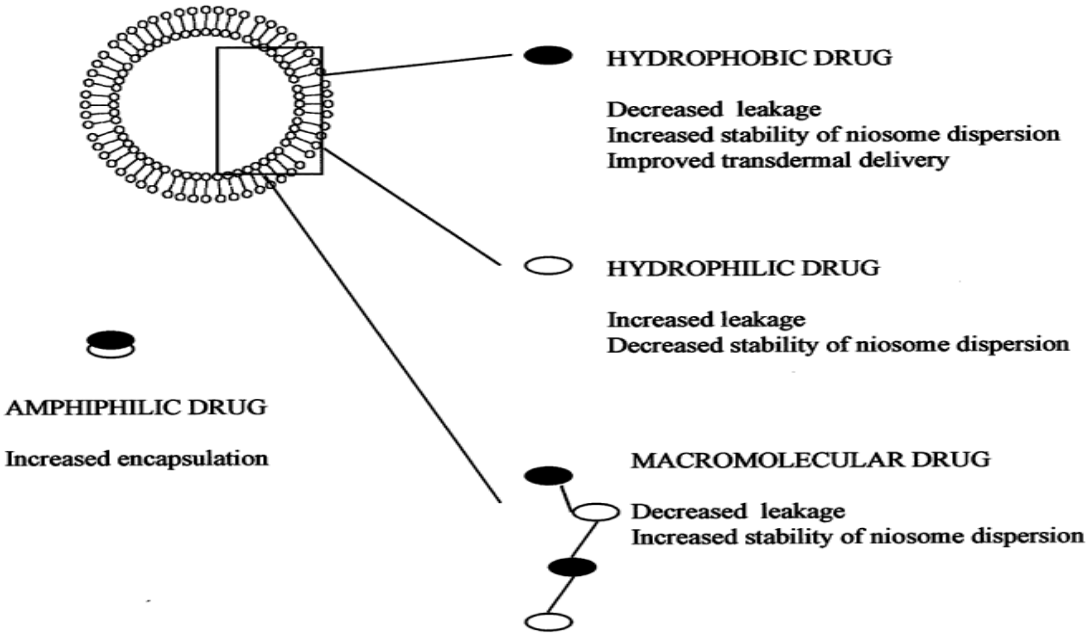


Figure 2.6 Effect of the nature of the encapsulated drug on the properties of the niosome dispersion

Table 2.1 Effect of drug nature on various properties

Nature of the drug	**Leakage from the vesicle**	**Stability**	**Other properties**
Hydrophobic drug	Decreases	Increases	Improved transdermal delivery
Hydrophilic drug	Increases	Decreases	-
Amphiphilic drug	Decreases	-	Increased encapsulation, altered electrophoretic mobility
Macromolecules	Decreases	Increases	-

2.4.3 Methods of preparation of niosomes [14,17–19]

- **Hand Shaking Method**

Surfactant+cholesterol+solvent

↓

Remove organic solvent at room temperature

↓

Thin layer formed on the walls of flask

↓

Film can be rehydrated to form multi-lamellar niosomes.

- **Microfludisation**

Two Ultra high speed jets inside interaction chamber

↓

Impingement of thin layer of liquid in micro channels

↓

Formation of uniform niosomes

- **Reverse Phase Evaporation Technique**

Cholesterol+surfactant dissolved in ether+chloroform

↓

Sonicated at 5^{o}c and again sonicated after adding PBS

↓

Drug in aqueous phase is added to above mixture

↓

Viscous niosome suspension is diluted with PBS

↓

Organic phase is removed at 40 ^{o}C at low pressure

↓

Heated on a waterbath for 60 ^{O}C for 10 min to yield niosomes

- **Ether injection method**

Surfactant is dissolved in diethyl ether

↓

Then injected in warm water maintained at 60 ^{o}C through a 14 gauze needle

↓

Ether is vaporized to form single layered niosomes

- **Trans membrane pH gradient (insideacidic) Drug Uptake Process (remote Loading)**

Surfactant and cholesterol are dissolved in chloroform, which is then evaporated from round bottom flask

↓

Hydrated with 300 mM citric acid (pH 4.0) by vortex mixing

↓

Multilamellar vesicles are frozen and thawed 3 times and later sonicated

↓

Aqueous solution of drug is added and pH is set to 7.0-7.2 with disodium phosphate

↓

Mixture is later heated at 60°C for 10 minutes to give niosomes

- **Bubble Method**

RBF as bubbling unit with three necks in water bath

↓

Reflux, thermometer and nitrogen supply by three necks

↓

Cholesterol+surfactant dispersed in buffer pH 7.4 at 70^{o}C

↓

Above dispersion is homogenized for 15 sec and then bubbled with nitrogen gas at 70^{o}C to get niosomes

- **Sonication method**

Drug in buffer+surfactant/cholesterol in 10 ml

↓

Above mixture is sonicated for 3 min at 60oC using titanium probe yielding niosomes

2.4.4 Post-Preparation Processes [13,20]

The main post-preparation processes in the manufacture of niosomes are downsizing and separation of unentrapped material. After preparation, size reduction of niosomes is achieved using one of the methods given below:

1. Probe sonication results in the production of the niosomes with smaller size.
2. Extrusion through filters of defined pore sizes could reduce size of vesicles.
3. Combination of sonication and filtration could be used to obtain niosomes in the 200nm size range.

4. Microfluidization yields niosomes in below 100 nm size.
5. High-pressure homogenization also yields vesicles of below 100nm in diameter.

- As in most cases 100% of the drug cannot be encapsulated in the niosomal vesicles, the unentrapped drug should be separated from the entrapped ones.
- Most commonly used methods for separating unentrapped material from niosomes are as follows:
  - Dialysis
  - Gel filtration (e.g. Sephadex G50); or separation by solid phase extraction (SPE)
  - Centrifugation (e.g. 7000 × *g* for 30 min for the niosomes prepared by handshaking and ether injection methods)
  - Ultracentrifugation (150000 × *g* for 1.5 h)

2.4.5 Stability of niosomes [21, 22, 23]

Vesicles are stabilized based upon formation of 3 different forces:

1. Van der Waals forces among surfactant molecules
2. Repulsive forces emerging from the electrostatic interactions among charged groups of surfactant molecules or charge inducer
3. Entropic repulsive forces of the head groups of surfactants.

2.4.6 Factor affecting stability of Niosomes

The factors which affect the stability of niosomes are as follows:

- Type of surfactant
- Nature of encapsulated drug
- Storage temperature
- Use of membrane spanning lipids;
- The interfacial polymerization of surfactant monomers in situ;
- Inclusion of a charged molecule.

2.4.7 Advantages of niosomes [11,13,17,21]

- The vesicle suspension being water based offers greater patient compliance over oil based systems
- Since the structure of the niosome offers place to accommodate hydrophilic, lipophilic as well as amphiphilic drug moieties, they can be used for a variety of drugs.
- The characteristics such as size, lamellarity etc. of the vesicle can be varied depending on the requirement.
- The vesicles can act as a depot to release the drug slowly and offer a controlled release.
- They are osmotically active and stable.
- They increase the stability of the entrapped drug
- Handling and storage of surfactants do not require any special conditions
- Can increase the oral bioavailability of drugs
- Can enhance the skin penetration of drugs
- They can be used for oral, parenteral as well topical.
- The surfactants are biodegradable, biocompatible, and non-immunogenic.
- Improve the therapeutic performance of the drug by protecting it from the biological environment and restricting effects to target cells, thereby reducing the clearance of the drug.
- The niosomal dispersions in an aqueous phase can be emulsified in a non-aqueous phase to control the release rate of the drug and administer normal vesicles in external non-aqueous phase.

2.4.8 Disadvantages of niosomes

- Physical instability
- Aggregation
- Fusion
- Leaking of entrapped drug
- Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion (for hydrophilic drugs)

2.4.9 Applications of niosomes [4,22,23,24]

1. Transdermal Applications

It is well-known fact that transdermal applications provide a great advantage of protecting drugs from the hepatic first pass effect. However, stratum corneum layer of skin forms a barrier, resulting in a slow absorption at the application site. The fact that in the manufacturing of niosomes nonionic surfactants are used makes them good candidates for transdermal drug delivery.

Two mechanisms are suggested for transdermal absorption of vesicles:

1. Diffusion of nisomes from the stratum corneum layer of skin as a whole
2. Forming new vesicles by each individual component (re-formation of vesicles).

The later one takes place only at certain regions of stratum corneum where water content is high. Many researchers agree upon the second mechanism since the diameter of vesicles is larger than the lipid lamellar spaces of the stratum corneum. Figure 2.7 shows various mechanisms of actions.

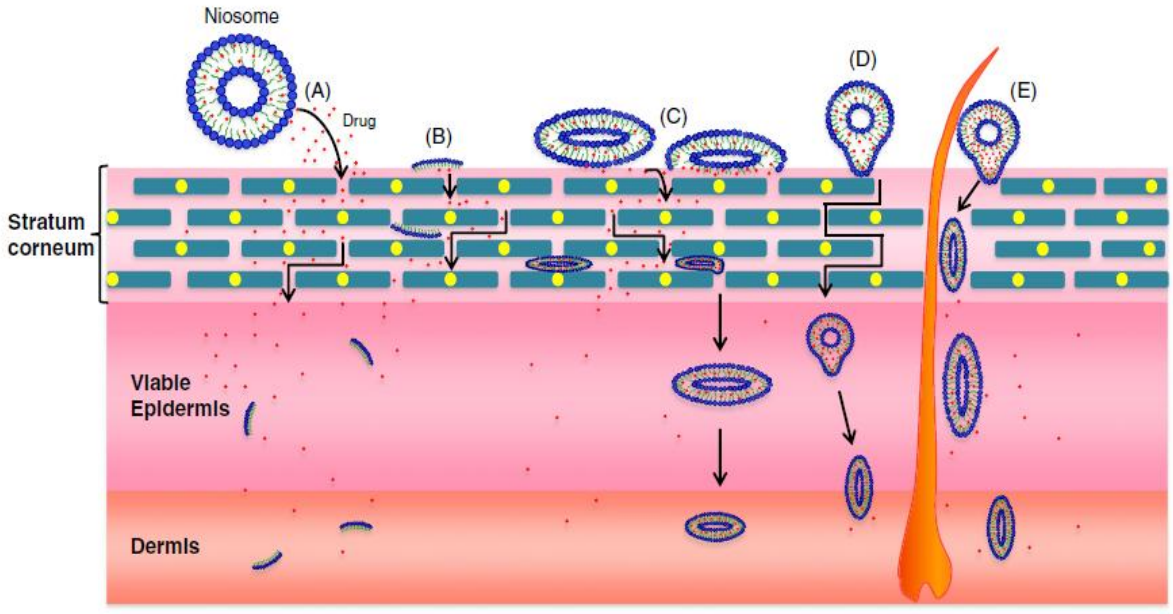


Figure 2.7: Possible mechanisms of action of surfactant vesicles for dermal and transdermal applications: (A) drug molecules are released by niosomes, (B) niosome constituents act as penetration enhancer, (C) niosome adsorption and/or fusion with Stratum Corneum; (D) intact niosome penetration through the intact skin; (E) niosome penetration through hair follicles and/or pilosebaceous units.

2. Ophthalmic drug delivery

It is difficult to achieve excellent bioavailability of drug from ocular dosage form like ophthalmic solution, suspension and ointment due to the tear production, impermeability of corneal epithelium, non-productive absorption and transient residence time.

3. Parenteral Applications

Niosomes in sub-micron size are used for parenteral administration. Niosomal vesicles up to 10 μm are administered via i.p. or i.m.

4. Oral Applications

The oral use of niosomal formulations was first demonstrated by Azmin et al (1985) in a study involving 100 nm methotrexate C16G3 niosomes. Significantly higher levels of methotrexate were found in the serum, liver and brain of PKW mice following oral administration of a niosomal formulation. It thus appears that there is enhanced drug absorption with these niosomal formulations.

5. Radiopharmaceuticals

The first applications of niosomes as radiopharmaceuticals have been achieved by Erdogan et al in 1996. They prepared 131I labeled iopromide niosomes with positive charge in order to enhance contrast during CT in rats (Erdogan et al 1996). The formulations were in the form of gel or liquid crystal. They were found more in kidneys and maintained their activity over 24 hours. In another study, Korkmaz et al (2000) used 99mTc- labeled DTPA containing niosomes and found that DTPA was accumulated in liver and spleen in large quantities. The gamma sintigraphic images of mouse were better with 99mTc-DTPA niosomes [N1 formulation: SurI: SA: CHOL (10:1:4)]. Similarly, gel type 99mTc-labelled niosomes of DMSA accumulated in liver, kidneys, and spleen in mouse and maintained the activity for 24 hours. Niosome formulation also provided better stability in comparison to conventional solutions of DMSA as they are less susceptible to light, temperature and oxidation.

6. Ophthalmic Drug Delivery

There is only a single study on the use of niosomes for ophthalmic drug delivery to date (Saettone et al 1996). Saettone et al (1996) reported on the biological evaluation of a niosomal Cyclopentolate delivery system for ophthalmic delivery. Polysorbate 20 and cholesterol were used for niosome formulations. It was determined that cyclopentolate penetrated the cornea in a pH dependant manner within these niosomes. Optimum pH for peak permeation values was pH 5.5. Permeation decreased at pH 7.4. However, in vivo data revealed that there was increased mydriatic response with the niosomal formulation irrespective of the pH of the formulation. In short, the increased absorption of cyclopentolate may be the result of the altered permeability characteristics of the conjuctival and scleral membranes. Niosomes >10 μm are suitable for drug administration to eye.

2.5 Introduction to 2^3 full factorial design [25]

Design of experiments should be implemented as it helps in finding the optimized batch with minimum number of trials. There are several designs which could be applied depending on the factors and levels of the study. Out of various designs of experiments, a 2^3 full factorial design was selected to study the influence of several operational parameters on the final formulation. Hence, the resulting experimental design consisted of 8 runs with three central batches (optional), hence total of 11 runs are required. A series of experimental trials were performed and evaluated based on the setup of experimental runs taken at different combinations of factor levels. All the values of responses were fitted to a linear equation model and the adequacy of this model was checked by various statistical parameters such as ANOVA, lack of fit, and multiple correlation coefficients R^2 tests. The linear equation model is represented as below.

$$Y = b0 + b1A + b2B + b3C + b12AB + b23BC + b13AC.$$

Where, y = dependent variable or measured response,
b1, b2, b3 are the estimated coefficients for the factors A, B, C respectively b0 is the intercept. The coefficient of responses exemplifies the relative importance of each factor. An optimized formulation can be found out from the overlay graph obtained from design expert software. Formulation having optimum response features based on the specifications required is then chosen.

2.5.1 Advantages of factorial designs

1. For each independent variable minimum numbers of trials are required.
2. Among various other designs, factorial designs have maximum efficiency in finding out the main effects.
3. They form the basis of various other designs such as CCD, fractional factorial design etc.
4. They are the basic unit in defining a large response surface.
5. The responses are estimated with maximum precision.
6. By these designs both quantitative and qualitative variables can be tested and interpretation of results can be done easily.

2.5.2 Applications

1. Design helps in interpreting the mechanism of an experimental system.
2. It suggests and instigates a practical procedure or set of experimental condition in the pharmaceutical industries.
3. It provides guidance for further experimentations which are to be done in order to maximum results with minimum number of trials.

2.5.3 Introduction to concept of desirability function

Responses should be highly correlated with each other during the optimization process. The values that are desirable to optimize the effect of one response might not have the same effect on the second response; hence a clash can occur between them and in order to avoid this conflict the most favourable compromising zone must be sought for each of the responses without any prejudice. In case of multiple responses each response were simultaneously optimized by a desirability function that incorporates the numerical optimization method discovered by Derringer and Suichin the design expert software version 9 (Stat-Ease Inc.)

In the desirability function, each response has a particular desired goal and multi-criteria responses are treated as single criterion problems. Individual response is accompanied with its partial desirability function where,

1. Value 0 (Zero) is assigned to an unacceptable response
2. Value between 0 and 1 is assigned to an acceptable response

The more close the response to the target value, considered as the least to the most desirable. A criterion for considering any response totally unacceptable is its falling outside the desirability limit. In order for the response to be maximized, the desirability function can be stated as:

$$\mathbf{D_i\ max = R_i - R\ min / R\ max - R\ min}$$

Where,

D_i max = Individual desirability of the responses to be maximized,

R_i = Experimental result, and R min and R max represent the minimum and maximum possible values.

If R_i is equal to or less than R min, then D_i max = 0, and if R_i is higher or equal to R max, than D_i max = 1.

In order for the response to be minimized the desirability function is stated as:

$$\mathbf{D_i\ min = R\ max - R_i / R\ max - R\ min}$$

Where,

If R_i is greater than R max, then D_i min = 0;

If R_i is less than or below the minimum, then D_i min = 1.

After obtaining the individual desirability values for each response, the results are usually combined as a geometric mean to give a global desirable value (D), which is described by equation below:

$$\mathbf{D = (D1*D2*D3*D4*\ldots\ldots\ldots\ldots*Dn)\ 1/n = (D_i)\ 1/n}$$

Where,

n specifies the number of responses being optimized.

According to the simultaneously assigned goals for all responses, the design expert software version 9 (Stat-Ease Inc.) determines the maximum desirability value by an extensive grid search over the domain.

2.5.4 Interpretation of data: Done by Response surface graphs using Design expert software version 9: (3-D plot & contour plot analysis)

- All the batches were evaluated on the basis of various parameters performed and their analysis was done by plotting contour plot& 3-D plot using various statistical parameters such as ANOVA, regression, p value etc.

- These types of plots are very useful for studying the interaction effects between two factors and for understanding how the effect of one factor will be influenced by the change in the level of another factor.
- As these types of plots can only express two independent variables at a time against the response, one independent variable must always be fixed.

2.6 Introduction to Drug (gamma oryzanol)

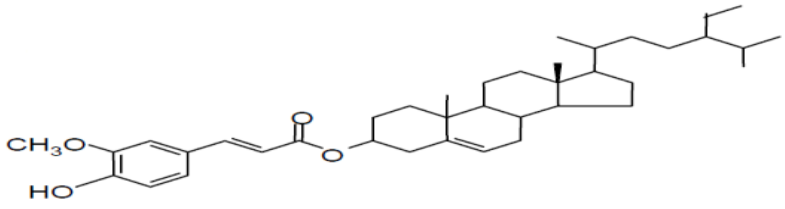
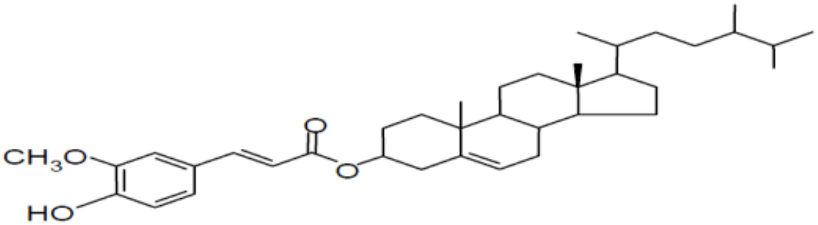
2.6.1 Source of gamma Oryzanol [18,26–30]

Crude rice bran (CRB), a by-product of rice milling, is rich in phytochemicals of high nutritional value, such as Gamma-oryzanol (OZ), tocopherols and tocotrienols. Gamma Oz was first isolated from rice bran oil in 1954. OZ consists of a mixture of ester compounds derived from the reaction of trans-ferulic acids with phytosterols and triterpene alcohols.

Commercial rice bran oil (RBO) is obtained from CRB through a process involving two major operations: (i) solvent extraction of CRB to obtain RBO and subsequent solvent distillation, and (ii) refining of the obtained RBO. Rice bran oil (RBO) is the most abundant natural source of OZ with overall yields of between 1.8 and 3.0 %

Initially, OZ was thought to be a single compound but it is known now that OZ is a mixture of 10 components that consist of ferulic acid and triterpene derived compounds combined by an ester bond. After that several other major constituents and their derivatives were extracted from rice. The four major components of gamma-oryzanol were determined to be cycloartenylferulate, 24- methylenecycloartanylferulate, campesterylferulate, and sitosterylferulate, out of this first three are major components and it accounts for 80 % of OZ in rice bran oil.The structures of major components are as shown in table 2.2.

Table 2.2 structures of major component of OZ

Sitosterylferulate	
Campesteryl ferulate	

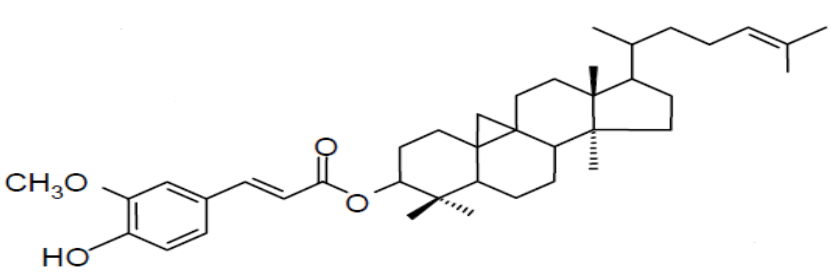
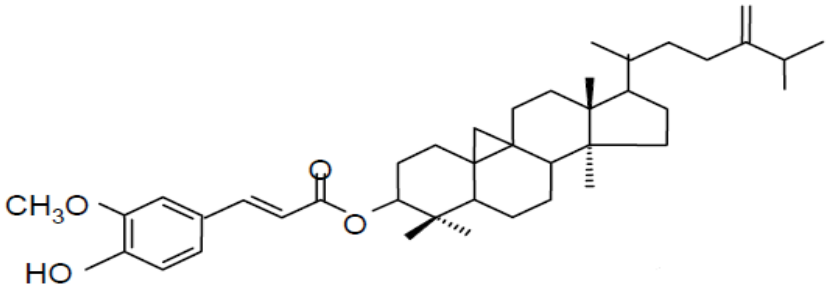
Cycloartenyl ferulate	
24-methylene cycloartenol ferulate	

Table 2.3 Physico-chemical Properties of drug (gamma oryzanol) [25, 26, 27]

PROPERTIES	**DESCRIPTION**
Chemical name	Gamma oryzanol
Physical state	Solid; (Powdered solid)
Odour and Colour	Odourless and white in colour
Solubility	Soluble in acetone, heptane, ether, chloroform, benzene, ethanol (Slightly soluble), and water (Very slightly soluble).
Log P	10.12
Density	1.11 g/cm3
Melting Point and Boiling Point	160-172 °C and 663.22 °C at 760 mmHg

IUPAC Name	(1S,3R,6S,8R,11S,12S,15R,16R)-7,7,12,16-tetramethyl-15-[(2R)-6-methylhept-5-en-2yl]pentacyclo[9.7.0.0^47.0^47.0^1]octadecan-6-yl(2E)-3-(4-hydrxy-3-methoxyphenyl)prop-2-enoate
Synonyms	Gammariza; Gamma-orizanol, HI-Z; OZ; Oliver; gamma-OZ
Molecular refractivity	180.045
Molecular formula	C40H58O4
Molecular weight	602.98
pKa	9.74
λmax in nm	310-330 (vary with solvents) Ethanol- 326 PBS 7.4 - 327
Storage	Store at 4° C, Store under room temperature in dark place in sealed condition, Avoid places with high temperature and high humidity
Dosage Strengths	50mg thrice a day, 300 mg per day, taken in divided doses (i.e. 150 mg, twice per day), 500mg/day
Marketed formulations	**Gammanol Forte with FRAC** from Biotics Research Corp.

2.6.2 Therapeutic Indications, Therapeutic Index and Toxicity [27]

OZ's physiological functions are associated with decreasing plasma cholesterol, lowering serum cholesterol, decreasing lipid absorption, decreasing platelet aggregation. It has been used to treat hyperlipidemia, atherosclerosis, suppression of inflammation, diabetes, allergy, to increase muscle mass. It is found to have positive effect on stress related

disorders & offers various beneficial effects in terms of improvement of dry skin, skin whitening effect & addressing menopausal syndrome. It is also used in Japan to treat psychosomatic disease as it involves in the metabolism of catecholamine such as dopamine, adrenaline and nor-adrenaline in the hypothalamus. On the other hand, OZ is hydrolyzed almost completely in the process of digestion in the body. I.P administration of gamma OZ 10,000 mg/kg showed no abnormality generally as reported by scientists. Similarly, no abnormality was observed on subcutaneous administration of 500 mg/kg OZ.

2.6.3 Pharmacokinetic Properties (absorption, distribution and metabolism) [28]

Plasma concentration of metabolite of OZ was found to reach its peak levels at 4 to 5 hours post administration and decreasing rapidly to certain level and remained for 48 hours in rabbits. Meanwhile, 5-10% of metabolites found in the urinary excretion and 17-32% in the faeces respectively after 48-hour administration. In terms of distribution, OZ was found to be largely distributed in the brain with its metabolite uniformly distributed among organs and largely accumulated in the liver however less in the reproductive organs. However, single dose administration did not reveal a large distribution in the brain; continuous administration may result in 5-10 folds of distribution.

2.6.4 Enzymatic interaction [29]

Gamma-Oryzanol, at 1-30 µg/ml, failed to significantly inhibit CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4; with the most inhibitory potential being a mere 16% at 30 µg/ml on CYP1A1/2.

CHAPTER 3

LITERATURE REVIEW

3. LITERATURE REVIEW

3.1 Literature review on Transdermal vesicular drug delivery system [30-35]

1. Glavas-Dodov et al. Liposome gels bearing an antineoplastic agent, 5-Fluorouracil, intended for topical application have been prepared. Liposomes were prepared by the film hydration method by varying the lipid phase composition (PL 90H/cholesterol mass ratio) and hydration conditions of dry lipid film (drug/aqueous phase mass ratio). Topical liposome gels were prepared by incorporation of lyophilized liposomes into a structured vehicle (1%, m/m, chitosan gel base). The rate of drug release from liposome gels was found to be dependent on the bilayer composition and the dry lipid film hydration conditions. The drug release obeyed the Higuchi diffusion model, while liposomes acted as reservoir systems for continuous delivery of the encapsulated drug.

2. Dubey et al. Melatonin loaded liposomes were prepared and were found to bispherical, unilamellar structures having low polydispersity (0.032 ± 0.011) and nanometric size range (122 ± 3.5 nm). % entrapment efficiency of drug in ethosomal carrier was found to be 70.71 ± 1.4. Stability profile of prepared system assessed for 120 days revealed very low aggregation and growth in vesicular size (7.6 ± 1.2%). MT loaded ethosomal carriers also provided an enhanced transdermal flux of 59.2 ± 1.22 μg/ cm^2/ hr and decreased lag time of 0.9 hr across human cadaver skin.

3. Agarwal et al. Dianthrol was entrapped in vesicles to help in the localized delivery of the drug and to improve availability of the drug at the site to reduce the dose and, in turn, the dose-dependent side effects like irritation and staining. The mean liposome and niosomes sizes were 4 ± 1.25 and 5 ± 1.5 μm, respectively. The drug-leakage study carried out at different temperatures of 4–8, 25 ± 2, and 37 ^{0}C for a period of two months affirms that the drug leakage increased at a higher temperature. The *in vitro* permeation study using mouse abdominal skin showed significantly enhanced permeation with vesicles as indicated by flux of Dianthrol from liposomes (23.13 μg/cm^2/hr) and niosomes (7.78 μg/cm^2/hr) as compared with the cream base (4.10 μg/cm^2/hr).

4. Patel et al. Liposomes of Ketoconazole were prepared for topical application. The drug entrapment efficiency was found to be 54.41 ± 0.19%. The percentage cumulative drug release was determined by diffusion studies and found to be 34.96 ± 0.86% after 12 hours. Stability studies present percent drug retention at refrigerated temperature (2–8 ^{0}C).

5. Moghimipour et al. Liposomal gel of Triamcinolone acetonide were prepared to increase the deposition of drugs within the skin at the site of action and reduce side effects of drug using carbomer 940 as gelling agent. Four different gel formulations including hydroalcoholic, multilamellar large vesicles (MLV), small unilamellar vesicles (SUV), and blank MLV gel containing free drug were prepared. The *in vitro* drug release studies were determined using dialysis membrane method. The results of drug release showed that SUV liposomal gel has the most regular and the least interaction between the drug and polymer.

6. Hundekar et al. Transdermal cubosomes of Diclofenac sodium were prepared to increase the percutaneous absorption with reduced systemic side effects. It was concluded that the Diclofenac sodium cubosome gel shows enhanced drug penetration through Wistar albino rat skin *in vitro* and *in vivo* and cubosome gel containing Diclofenac sodium may offer promise as an anti-inflammatory dosage form.

3.2 Literature review on Niosomal drug delivery system [36-43]

1. Parthibarajan et al. Voriconazole niosomes were prepared by hand shaking and ether injection method using span 80 and cholesterol. The niosomes size range was 0.5–5 μm and 0.5–2.5 μm by hand shaking method and ether injection method, respectively. The percentage entrapment efficiency of niosomal formulation containing Voriconazole was determined by dialysis method. The % drug entrapment efficiency was found to be more than 84.53% in case of niosomes prepared by hand shaking process. The *in vitro* release profile of drug indicated 70.06% drug release extended period of 24 hours for the formulation prepared by hand shaking method.

2. Allam et al. Ophthalmic niosomes of Acyclovir were prepared using two different methods, that is, film hydration method (FHM) and reverse phase evaporation method (REV). Particle size distribution studies showed that particle size was the smallest in absence of cholesterol and as the amount of cholesterol was increased, subsequent increase in particle size was observed. Corneal permeation studies showed that the cumulative amount permeated from most niosomal formulations was lower than that from solution containing drug, except the formulations having cholesterol: surfactant molar ratio 1: 1. No corneal damage was observed during corneal hydration studies. Prepared niosomes were more stable at 4 ^{0}C than at 25 ^{0}C.

3. Sathyavathi et al. Niosomal *in-situ* gel of Brimonidine tartrate was developed using different ratios of span series and cholesterol for improved ocular bioavailability for the treatment of glaucoma. Small uni-lamellar vesicles were prepared in the size range of 50–100 nm. The niosomal formulation was transformed into gel when it was instilled into the eye. All the formulations exhibited pseudo-plastic rheological behaviour and slow drug release pattern. Anti-glaucoma activity of the prepared gel formulations showed more significant and sustained effect in reducing intraocular pressure than marketed and niosomal drops.

4. Rani et al. Niosomes of rifampicin and gatifloxacin were prepared by lipid hydration technique using rotary flask evaporator. The prepared niosomes showed a vesicle size in the range of 100–300 nm, the entrapment efficiency was 73% and 70%, respectively. The

in vitro release study showed 98.98% and 97.74% of release of rifampicin and gatifloxacin niosomes, respectively. The bactericidal activities of prepared formulation were studied by BACTEC radiometric method using the resistant strains (RF 8554) and sensitive strains (H37Rv) of *Mycobacterium tuberculosis* which showed greater inhibition and reduced growth index.

5. Ning et al. Niosomes were evaluated as delivery vehicles to develop alternative formulation for the vaginal administration of clotrimazole, to provide sustained and controlled release of appropriate drug for local vaginal therapy. Niosomes were prepared by lipid hydration method and were incorporated into 2% carbopol gel, and the systems were evaluated for drug stability in phosphate-buffered saline (pH 7.4) and simulated vaginal fluid at 37 ± 1 ^{0}C. Further, the vesicle gel system was evaluated by antifungal activity and tolerability on tissue level in rat.

6. Mura et al. Minoxidil loaded niosomes were formulated to improve skin drug delivery. Multilamellar niosomes were prepared using soya phosphatidylcholine. Minoxidil skin penetration and permeation experiments were performed *in vitro* using vertical diffusion franz cells and human skin treated with either drug vesicular systems or propylene glycol-water-ethanol solution (control). Penetration of Minoxidil in epidermal and dermal layers was greater with liposomes than with niosomal formulations and the control solution. The greatest skin accumulation was always obtained with non dialyzed vesicular formulations. No permeation of Minoxidil through the whole skin thickness was detected in the present study. It was concluded that alcohol-free liposomal formulations would constitute a promising approach for the topical delivery of Minoxidil in hair loss treatment.

7. Guinedi et al. Niosomes were prepared by reverse-phase evaporation and thin film hydration method using Span 40 or Span 60 and cholesterol in the molar ratios of 7: 4, 7: 6 and 7: 7. Stability studies were carried out to investigate the leaching of drug from niosomes during storage. It was observed that the type of surfactant, cholesterol content, and the method of preparation altered the entrapment efficiency and drug release rate from niosomes. Higher entrapment efficiency was obtained with multilamellar niosomes

prepared from Span 60 and cholesterol in a 7: 6 molar ratio. Niosomal formulations have shown a fairly high retention of Acetazolamide inside the vesicles (approximately 75%) at a refrigerated temperature up to a period of 3 months. Multilamellar Acetazolamide niosomes formulated with Span 60 and cholesterol in a 7: 4 molar ratio were found to be the most effective and showed prolonged decrease in intraocular pressure.

8. Ruckmani et al. Niosome vesicles of Cytarabine hydrochloride were prepared by a lipid hydration method that excluded dicetylphosphate. The sizes of the vesicles obtained ranged from 600 to 1000 nm; with the objective of producing more blood levels in vivo. The study of the release of drug from niosomes exhibited a prolonged release profile as studied over a period of 16 hr. The drug entrapment efficiency was about 80% with Tween 80, Span 60, and Tween 20; for Span 80, it was 67.5%.The physical stability profile of vesicular suspension was good as studied over a period of 4 weeks.

3.3 Literature review on Drug (Gamma oryzanol) [44-50]

1. Ji Soo Lee et al. developed g-oryzanol-loaded calcium pectinate microparticles reinforced with chitosan. Response surface methodology was used to optimize microparticle preparation conditions, including the ratio of pectin: gamma-oryzanol (OZ) (*X*1), agitation speed (*X*2), and the concentration of emulsifier (*X*3), for maximal entrapment efficiency (EE) of OZ-loaded Ca pectinate microparticles. The optimized values of *X*1, *X*2, and *X*3 were found to be 2.72:5.28, 1143.5 rpm, and 2.61%, respectively. Experimental results obtained for the optimum formulation agreed favorably with the predicted results, indicating the usefulness of predicting models for EE. In order to evaluate the effect of chitosan-coating and blending on the release pattern of the entrapped OZ from microparticles, chitosan-coated and blended Ca pectinate microparticleswere prepared. Release studies revealed that the chitosan treatments,
Especially the chitosan-coating, were effective in suppressing the release in both simulated gastric fluid (SGF) and intestinal fluid (SIF).

2. Aranya Manosroi et al. The objective of this study was to determine antioxidant activities [by in vitro ORAC (oxygen radical absorbance capacity) and ex vivo lipid peroxidation inhibition assay] and in vivo human skin hydration effects of gel and cream containing the rice bran extracts entrapped in niosomes. Gel and cream containing the rice bran extracts entrapped in niosomes showed higher antioxidant activity (ORAC value) at 20–28 _mol of Trolox equivalents (TE) per gram of the sample than the placebo gel and cream which gave 16–18 mol TE/g. Human sebum treated with these formulations showed more lipid peroxidation inhibition activity than with no treatment of about 1.5 times. The three different independent techniques including corneometer, vapometer and confocal Raman microspectroscopy (CRM) indicated the same trend in human skin hydration enhancement of the gel or cream formulations containing the rice bran extracts entrapped in niosomes of about 20, 3 and 30%, respectively. This study has demonstrated the antioxidant activities and skin hydration enhancement of the rice bran bioactive compounds when entrapped in niosomes and incorporated in cream formulations.

3. Somsuvra B. Ghatak et al. The current study was initiated to investigate the effects of oryzanol (OZ), a commercially important bioactive phytochemical isolated from crude

rice bran oil (cRBO) against experimental diabetes as well as the antioxidant potential of the drug. Oral administration of OZ (50 and 100 mg/kg) reduced the blood glucose level in normal and in STZ (45 mg/kg, intravenous) diabetic rats in both single and multidose study. Oxidative stress produced by STZ was found to be significantly reduced by OZ when compared to control rats, as evident from a significant decrease in the extent of lipid peroxidation (LPO) and increased levels of enzymatic anti-oxidants such as superoxide dismutase (SOD) and reduced glutathione (GSH) in the liver. The findings indicate that OZ possesses the potential to effectively ameliorate the oxidative stress induced by STZ and produce a reduction in blood glucose levels. However, further experiments at the clinical level are warranted to confirm the utility of OZ in the therapeutic management of diabetes mellitus

4. Nispa Seetapan et al. In the present study, gamma oryzanol was incorporated into glycerol behenate (Compritol 888 ATO) nanoparticles (SLNs) at 5 and 10% (w/w) of lipid phase. Increasing lipid phase concentration resulted in increased consistency and particle diameter of SLNs. Upon storage over 60 days at 4, 25 and 40°C, the instability was observed by rheological analysis for all samples due to the formation of gelation. Rheological measurement revealed the increase in storage modulus and critical stress during storage at all temperatures. However, at 40°C, the pronounced instability was observed from the highest increase in storage modulus and a formation of rod-like network structure from scanning electron micrographs. An increase in crystallinity, determined by differential scanning calorimetry, was also found during storage at all temperatures, confirming the instability of SLNs. Particle diameters and zeta potentials of both concentrations at all storage conditions failed to explain the observed instability. These investigations helped in developing formulations of solid lipid nanoparticles, which are optimized with respect to the desired rheological properties.

5. M. Tamagawa et al. studied the carcinogenic potential of g-oryzanol, a drug mainly used for the treatment of hyperlipidaemia, was studied in B6C3F, mice. Groups of 50 males and 50 females were fed a diet containing 0 (control), 200, 600 or 2000 mg g-oryzanol/ kg body weight/day for 78 weeks. No treatment-related changes were observed in general condition, body weight, food consumption, mortality, organ weight or

haematology. Histopathological examinations showed various turnouts in all groups, including the control group. In the control and 2000 mg/kg groups, relatively high tumour incidences were observed in the liver of males and in the haematopoietic organs of females. However, there was no statistically significant difference in the incidence of any tumours between the control and the 2000 mg/kg groups. The findings indicated that under the experimental conditions described gamma oryzanol was not carcinogenic in B6C3F1 in mice.

6. M. J. Lerma García et al. reviewed the analytical methods for characterisation and determination; influence of genetic and environmental factors on the composition of rice bran; extraction approaches, including supercritical CO2 and subcritical water; and biomedical and industrial applications, including food and pharmaceuticals and found that the unsaponifiable constituents of RBO include mainly tocols (vitamin E, 0.10–0.14%) and g-oryzanol (esters of trans-ferulic acid with sterols and triterpenic alcohols, 0.9–2.9%). Concentration ranges of g-oryzanol, tocopherols and tocotrienols found in rice bran and RBO from different varieties and geographical areas were summarised. Hence, focus was given on the compilation of important findings available on gamma oryzanol.

7. Michele D'Ambrosio et al. They have developed a method for validation for separation of important components of gamma oryzanol by NP-HPLC in separating g-oryzanol components and developed a validated method for its routine quantification. The analysis is performed on a cyanopropyl bonded column using the hexane/MTBE gradient elution and UV detection at 325 nm. The method allows: the separation of steryl ferulate, pcoumarate and caffeate esters, and the separation of cis- from trans-ferulate isomers, the splitting of steroid moieties into saturated and unsaturated at the side chain. The optimised method provides excellent linear response (R2 = 0.99997), high precision (RSD < 1.0%) and satisfactory accuracy (R⁄ = 70–86%). Hence, it was concluded that the established method presents the details of the procedure and the experimental conditions in order to achieve the required precision and instrumental accuracy. The method was found to be fast and sensitive as a result it proved to be a suitable tool for quality assurance and determination of gamma oryzanol.

3.4 Patent survey

Patent Number	Important Findings
US2010/0068264 A1	Norma Alcantar et al. Their invention relates to drug delivery systems. Specifically, the invention relates to controlling the release rate of a therapeutic drug using nanoparticle vesicles embedded in hydrogel networks. The release rate can be controlled to last from 24 hours to more than 3 months. These niosomes are targeted for ovarian cancer. The invention includes a drug delivery medium comprising at least one niosome embedded in a polymer hydrogel. The niosome is comprised of a hydrophobic bilayer defining an interior hydrophilic space with at least one hydrophobic drug integrated into the hydrophobic bilayer and at least one hydrophilic drug encapsulated in the interior hydrophilic space. The hydrogel properties are preselected in accordance with a desired release rate for the niosomes and may include biodegradability, cross-link density, and pH-sensitivity and temperature sensitivity. In addition to hydrogel properties, niosome constituents are preselected in accordance with a desired release rate for the encapsulated drug and may include surfactants such as, without limiting the scope of the invention, crown ether amphiphiles bearing a steroidal moiety, 1,2-dialkyl glycerol polyoxyethylene ether, hexadecyl poly-5-oxyethylene ether, hexadecyl poly-5-oxyethylene ether ($C_{16}EO_5$); octadecyl poly-5-oxyethylene ether ($C_{18}EO_5$); hexadecyldiglycerol ether ($C_{16}G_2$); sorbitanmonopalmitate (Span 40) and sorbitanmonostearate (Span 60), Solulan™ C24 (poly-24-oxyethylene cholesteryl ether), polysorbate 20, Span detergents, Brij detergents, such as Brij-35, and polyoxyethylene, and polysorbates. Other components of the niosomes include, cholesterols, and, optionally, negative charged molecules such as dicetyl

	phosphates, Cetyl sulphate, phosphatidic acid, phosphatidyl serine, oleic acid, palmitic acid. The niosome comprises of at least one type of cholesterol.
US2007/0212470	Reddy Sastry et al. suggested the nutritional value of fiber derived from stabilized rice bran as well as its use thereof for the prevention and treatment of several GI disorders, bowel disorders, as a laxative, in weight loss diets, as a therapeutic diet for reducing cholesterol and blood glucose levels, for dissolving and preventing kidney and gallstone formation, for cancer prevention and for cancer treatment.
US20070172520	Michael VanAuker et al. Studied a immunoniosome for targeted delivery of a bioactive agent (antibody or fragment) entrapped in to niosomal membrane comprising of polyoxyethylene sorbitan monostearate (non-ionic surfactant); in which a bioactive agent having specific affinity for a cell surface antigens well as it is covalently coupled to the non-ionic surfactant. A cyanuric chloride is a linker which couples the antibody or fragment to the nonionic surfactant molecule. The niosomes thus provide a composition that enhances internalization or retention of the bioactive agent into the cytoplasm of the cells of the target tissue by providing a high degree of target specificity. Furthermore, the membrane vesicle enhances the life of the therapeutic agent by preventing its degradation in the extracellular environment, while exhibiting lower toxicity than can occur with some liposomes. The niosomes of the present invention are thus particularly useful as vehicles for the delivery of therapeutics to specific target cells.
US2004/20080269184	Makota Yuasa et al. A niosome having a metalloporphyrin complex embedded therein comprising a cationized metalloporphyrin complex and a niosome-forming substance. The niosome having a metalloporphyrin complex embedded therein has an SOD activity, can interact with superoxide

	anionic radicals (O_2^-.) as a target, and can reduce these radicals without fail. The niosome having a metalloporphyrin complex embedded therein can reach cells in living bodies such as cancer cells due to properties of a niosome. Therefore, the niosome having a metalloporphyrin complex embedded therein can exhibit an excellent effect of treating cancer by reducing O2-.in cancer cells. In addition, since the effect is selective, the niosome can be used as a novel anticancer agent without side effects. Moreover, the niosome having a metalloporphyrin complex embedded therein can be retained in the blood while exhibiting a superior antioxidation effect. The niosome can thus protect living bodies from hindrance brought about by active oxygen species.
US/2002/6410762	Kasturl Venkata et al. The present invention relates to a process for the isolation of oryzanol from rice bran oil soap stock by (a) saponifying the oil present in soap stock with an alkali followed by neutralisation of the excess alkali, (b) converting the soap stock into anhydrous, porous soap noodles, (C) extracting the soap noodles with an organic solvent, (d) crystallising the crude unsaponifiable matter to remove impurities, (e) subjecting the residue to column chromatography to obtain an oryzanol rich fraction, and recrystallising the oryzanol rich fraction using an organic solvent to obtain pure oryzanol.
US/1999/5985344	Cherukuri et al. developed a simple and cost effective enrichment process for enhancing antioxidant content of rice bran oil from crude rice bran oil (cRBO) is described. The process comprises extracting the cRBO using alcohol at 25-77° C. Obtaining the enriched rice bran oil (ERBO) from alcohol extracts Which contain 74 to 300 percent more antioxidants than the starting cRBO. The anti-oxidant enriched rice bran oil is useful in pharmaceutical, therapeutic, and dietary preparations.

CHAPTER 4

EXPERIMENTAL WORK

4. EXPERIMENTAL WORK

4.1 Material and Equipments

Table 4.1 List of materials

Materials	Company name
OZ (OZ)	Tokyo chemical company (Japan)
Span- 60	Central Drug House Ltd., (India)
Dicetyl Phosphate (DCP)	Sigma Aldrich
Carbopol 940p	Central Drug House Ltd., (India)
Glycerol LR	Central Drug House Ltd., New Delhi
Methyl paraben AR	Central Drug House Ltd., (India)
Propyl paraben AR	Central Drug House Ltd., (India)
Triethanolamine	Central Drug House Ltd., (India)
Methanol AR	Merck specialities private Ltd., (India)
isopropyl alcohol LR	Central Drug House Ltd., (India)
n-hexane AR	Merck specialities private Ltd., (India)
Ethanol AR	Central Drug House Ltd., (India)
Chloroform AR	Merck specialities private Ltd., (India)
Acetone AR	Merck specialities private Ltd., (India)
Benzene AR	Merck specialities private Ltd., (India)
Diethyl ether AR	Merck specialities private Ltd., (India)
potassium dihydrogen phosphate	Central Drug House Ltd., (India)
Sodium Chloride	Central Drug House Ltd., (India)
Sodium hydroxide	Central Drug House Ltd., (India)
Distilled water	Home made

Table 4.2 List of Equipments

Equipments	Company name
Digital Electronic balance	Scale tech. (India)
Magnetic Stirrer with hot plate	EIE Instrument Pvt Ltd., Ahmedabad
Mechanical stirrer with digital speed regulator	Remi Motors (India)
Bath Sonicator	Trans-o-Sonic D-Compact, (India)
Cooling centrifuge	RemiInstruments BL 250 (India)
Rotary vacuum evaporator	Popular traders (India)
Hot air oven	EIE Instruments Pvt Ltd. (India)
pH meter	Analab scientific instruments, (India)
Diffusion apparatus	Orchid Scientifics.
UV visible spectrophotometer	Shimadzu corporation (Japan)
FT-IR (Fourier transform Infra-red) spectrophotometer	Jasco FTIR 6100 TYPE A (Japan)
Zeta sizer	Malvern ZS 200
SPE	C-18 Column
Probe sonicator	Trans-o-Sonic (India)

4.2 Identification of Drug (OZ):

4.2.1 Melting point Determination:

Description:

The melting point of a substance can be defined as a temperature at which it is converted from solid state to liquid or at which solid and pure liquid are at equilibrium. Its range can be defined as the temperature at which it begins to melt up to temperature at which it melts completely. If melting point of substance is not within its specified range, it indicates impurity in substance. Hence melting point of a compound is an important criterion for identification of purity. Melting point apparatus is used for determining the melting point of drug in present investigation.

Table 4.3Melting point of Gamma OZ

Melting point	Standard	Observed
	160 – 170 ^{0}C	162-168^{0}C

Result: The melting point of gamma OZ was found to be 162 ^{0}C.

Discussion: The melting point of drug was found to be in the range of standard value, hence it was concluded that the sample is having same physical property as standard drug.

4.2.2 UV Spectrophotometric analysis:

4.2.2.1 UV absorption maxima of gamma OZ in ethanol and PBS (Phosphate buffer saline) pH 7.4

UV scanning was done for 25 µg/ml drug solution from 400-200 nm in using Shimadzu UV 1800 double beam UV/Visible spectrophotometer. The maximum absorbance was found to be at 326nm. (Fig: 4.1) similarly, UV scanning was done for 10 µg/ml drug solution from 400-200 in phosphate buffer saline solution (PBS) pH 7.4 using Shimadzu UV 1800 double beam UV/Visible spectrophotometer. The absorption maximum was found to be 327. (Fig: 4.2)

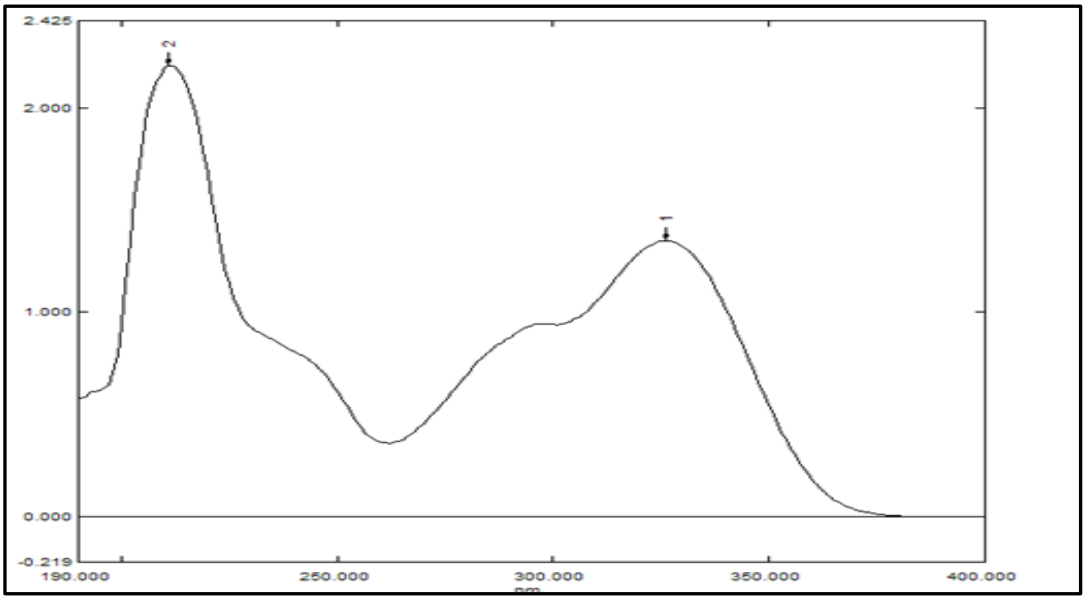


Figure 4.1 UV absorbance spectra of OZ (OZ) in ethanol

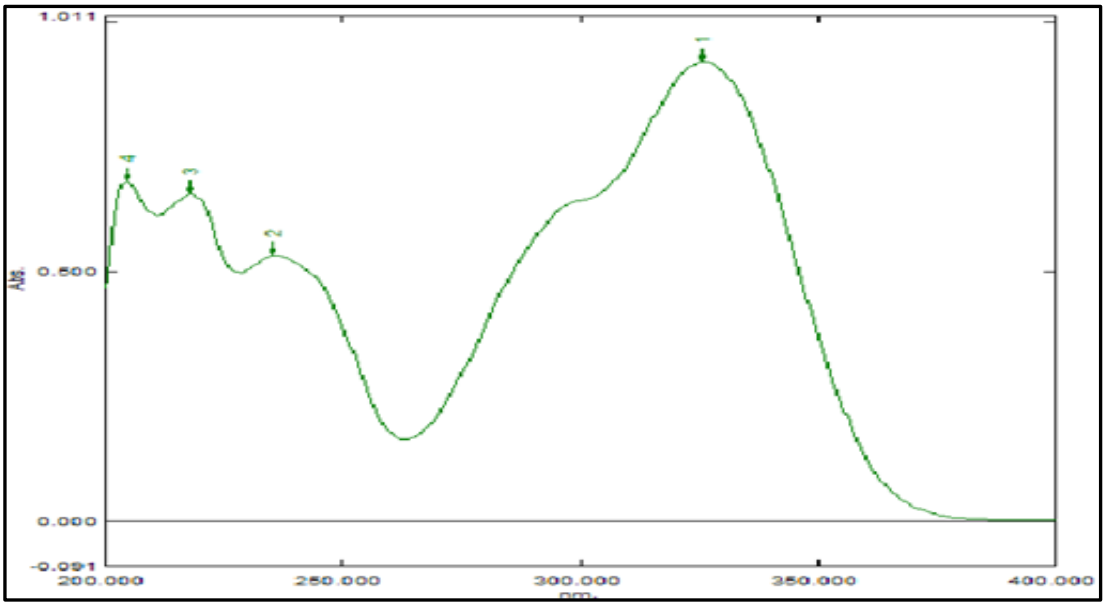


Figure 4.2: UV absorbance spectra of OZ (OZ) in PBS 7.4

- **Peak points**

Table 4.4 Absorption maxima of drug in ethanol and PBS 7.4

Solvent media	Wavelength (λ_{max}) in nm	Absorbance
Ethanol	326	1.394
PBS 7.4	327	0.11

Discussion: The above UV spectra of OZ showed the λmax at 326 nm in ethanol and 327nm in the PBS (phosphate buffer saline) pH 7.4, which was constantafter dilution and within range. This indicates authenticity and purity of the drug sample.

4.2.2.2 IR spectroscopy (FT-IR) analysis

The structure of the drug is mentioned in fig: 4.3 which indicated presence of various functional groups which can be identified by IR spectra. IR spectra of drug in KBr pellets at optimum scanning speed between 4000-400 cm^{-1} was carried out using FTIR (Jasco FTIR 6100 TYPE A, Japan) (Fig: 4.4) . All the powder samples were dried under vacuum prior to obtain the spectra to eliminate the effect of residual moisture present in the samples. The observed peaks were compared with standard. (Table: 4.5)

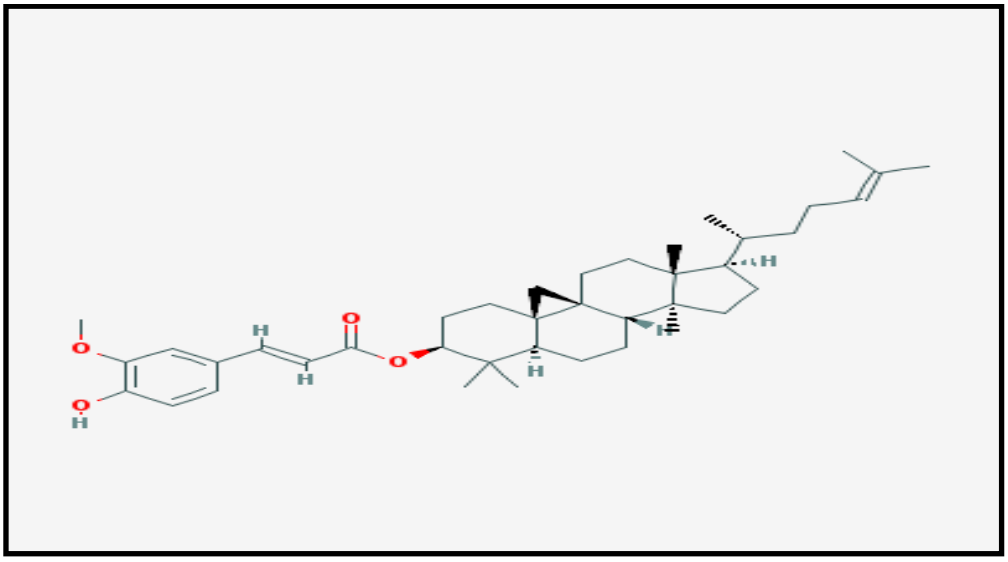


Figure 4.3 Chemical structure of OZ

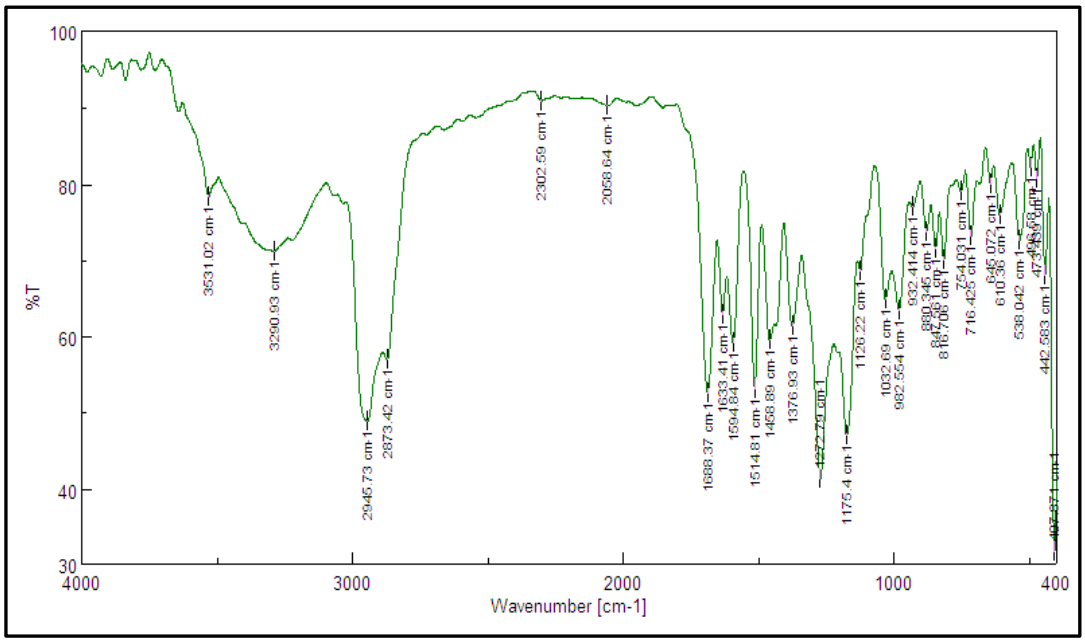


Figure 4.4 Infrared spectra of OZ

Table 4.5 Comparison of standard and observed frequencies of OZ (OZ)

Functional groups	**Standard frequencies (cm^{-1})**	**Observed frequencies (cm^{-1})**
Alkane (C-H Stretching)	2960-2850	2945.73
Aromatic hydrocarbon (C=C Stretching)	~1600, ~1450	1633.41, 1458.89
Alcohol (intermolecular H-bonded OH stretching)	3400-3200	3269.93
Ester(C=O stretching)	~1650	1688.37

Discussion: All the observed frequencies of functional group present in the drug was found to be matched with the standard IR frequencies of that functional group indicated that the drug was pure

4.3 Estimation of drug (OZ)

4.3.1 Standard curve of OZ in ethanol

- **Preparation of standard stock solution:**

  Ten mg of OZ was accurately weighed and transferred into 100 ml volumetric flask. Dilution was made up to 100ml mark with ethanol to obtain stock solution of 100µg/ml concentration.

- **Preparation of Standard Curve:**

  From the stock solution (100µg/ml), series of dilutions were prepared by withdrawing aliquots of 2, 4, 6, 8, 10 ml and transferredto volumetric flask. The final volume was made up to 10 ml with ethanol toget concentration in the range of 2-20 µg/ml. (Table: 4.6)

Table 4.6Standard curve of OZ in ethanol at λ max 326 nm

Concentration	Absorbance			Average ± SD
	I	II	III	
0	0	0	0	0
2	0.067	0.080	0.061	0.069 ± 0.0042
4	0.147	0.173	0.126	0.148 ± 0.0126
6	0.215	0.242	0.196	0.217 ± 0.0118
8	0.293	0.327	0.252	0.290 ± 0.0230
10	0.370	0.401	0.316	0.362 ± 0.0292
12	0.455	0.480	0.460	0.465 ± 0.0132
14	0.523	0.560	0.516	0.533 ± 0.0236
16	0.600	0.721	0.690	0.670 ± 0.0628
18	0.723	0.751	0.716	0.73 ± 0.0185
20	0.768	0.795	0.771	0.778 ± 0.0147

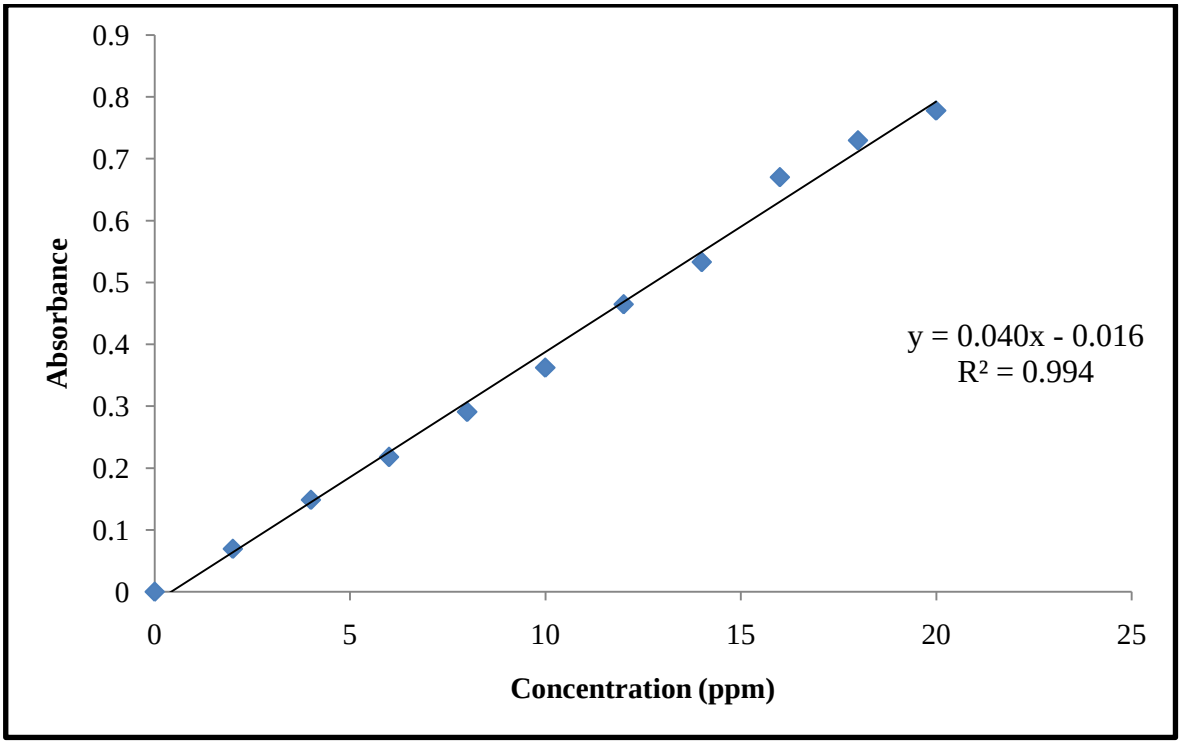


Fig 4.5 Standard curve of gamma OZ in Ethanol

Regression analysis

Table 4.7Regression analysis for standard curve of OZ in ethanol

Regression parameters	Values
Correlation coefficient	0.994
Slope	0.041
Intercept	0.024

4.3.1 Standard curve of OZ in PBS 7.4

- **Preparation of standard stock solution:**

  Twenty mg of OZ was accurately weighed and transferred into 100 ml volumetric flask. Dilution was made with PBS up to 100 ml mark to get 200µg/ml concentration.

- **Preparation of Standard Curve:**

  From the stock solution, a series of dilutions were prepared by withdrawing aliquots of 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 2.75, 3, 3.25, 3.5 ml from stock solution (200µg/ml) and were transferred to a 10 ml volumetric flask. The final volume was made up to 10 ml with PBS 7.4 to get concentration in the range of 5-70 µg/ml respectively. (Table: 4.8)

Table 4.8: Standard curve OZ in PBS at λmax327 nm

Concentration	Absorbance			Average ± SD
	I	II	III	
0	0	0	0	0
5	0.054	0.052	0.061	0.055± 0.0047
10	0.100	0.110	0.160	0.123± 0.0321
15	0.180	0.190	0.20	0.190± 0.0100
20	0.251	0.249	0.210	0.236 ± 0.0231
25	0.320	0.310	0.350	0.326 ± 0.0200
30	0.375	0.348	0.370	0.364 ± 0.0143
35	0.460	0.420	0.480	0.453 ± 0.0305
40	0.520	0.560	0.540	0.540± 0.0200
45	0.542	0.570	0.590	0.567 ± 0.0241
50	0.647	0.621	0.630	0.632 ± 0.0312
55	0.710	0.720	0.750	0.726 ± 0.0208
60	0.770	0.740	0.790	0.766 ± 0.0251
65	0.850	0.850	0.860	0.853 ± 0.0577
70	0.910	0.940	0.930	0.926 ± 0.0155

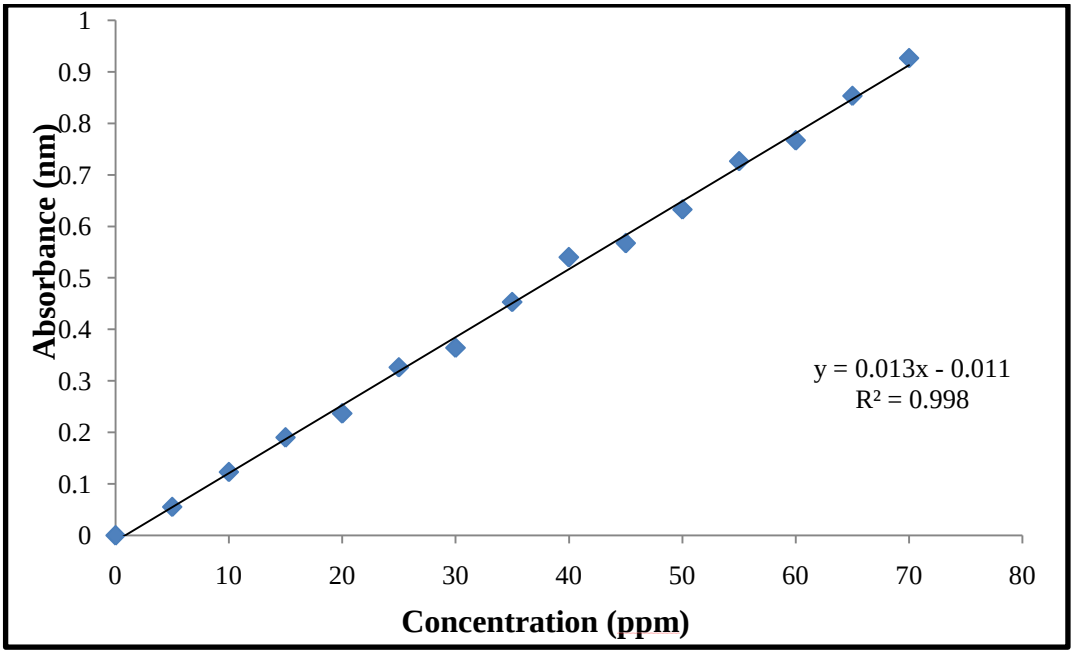


Fig 4.6 Standard curve of OZ in PBS 7.4

Regression analysis

Table 4.9Regression analysis for standard curve of gamma OZ in ethanol

Regression parameters	Values
Correlation coefficient	0.998
Slope	0.031
Intercept	0.011

Conclusion: As per Beer Lambert's law, the linearity range was found to be in between0-20 µg/ml in Ethanol and 0-70µg/ml in PBS 7.4. R^2 value was found within 1 which indicated good linearity. (Fig: 4.5 & 4.6)

4.4 Drug excipients compatibility study:

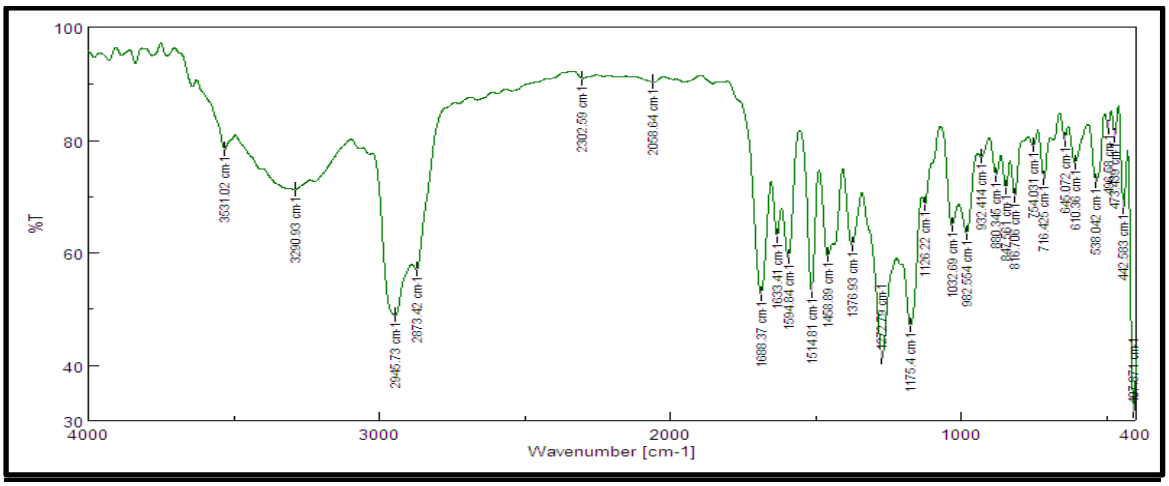
Drug excipients compatibility studies were performed using FT-IR spectroscopy. The IR spectra of pure drug and physical mixture of drug and excipients were studied by making the KBr mixture pellet. The principle absorption peaks of gamma OZ were obtained at different wave number in different sample. Samples were analyzed after one week and month stored at room temperature. The study was done using following drug excipients compatibility format. (Table: 4.10)

Table 4.10 Drug excipients compatibility study

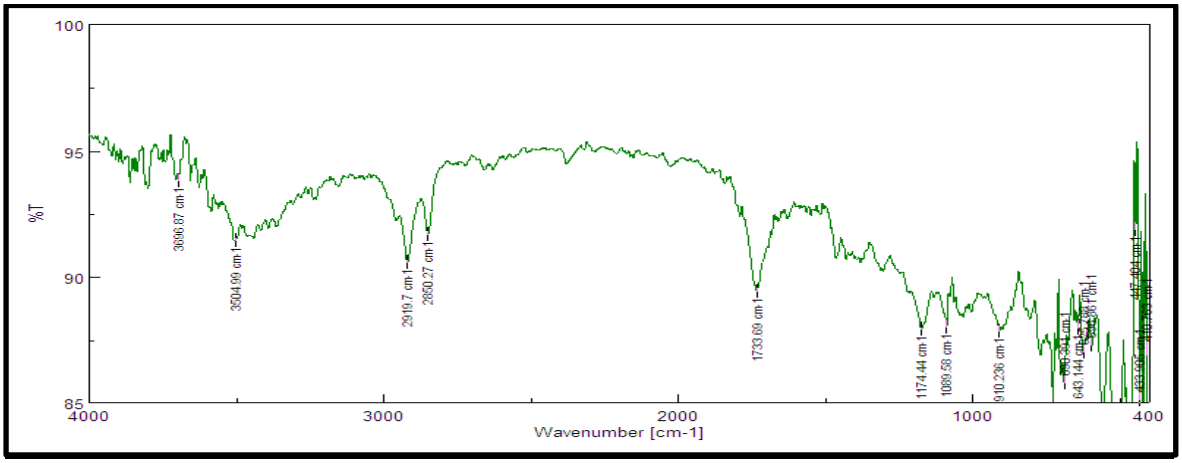
Sr. No.	Drug/Excipients	Time point	
		Initial	One month
1	Drug	1	1
2	Span 60	1	1
3	DCP	1	1
4	Drug + Span 60	1	1
5	Drug + DCP	1	1
6	Drug + span 60 + DCP	1	1

A) Observed IR spectra at initial time point

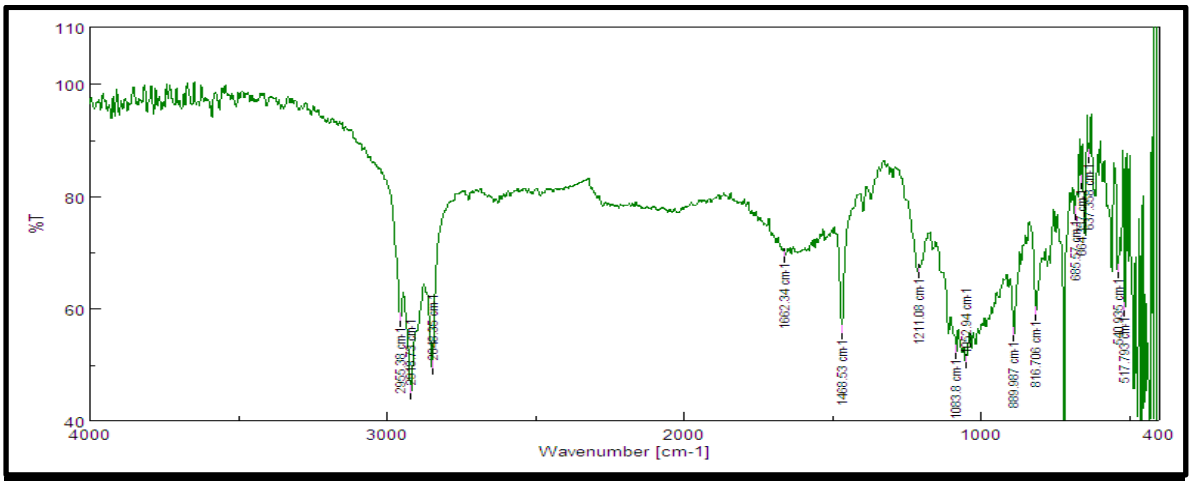
(i)



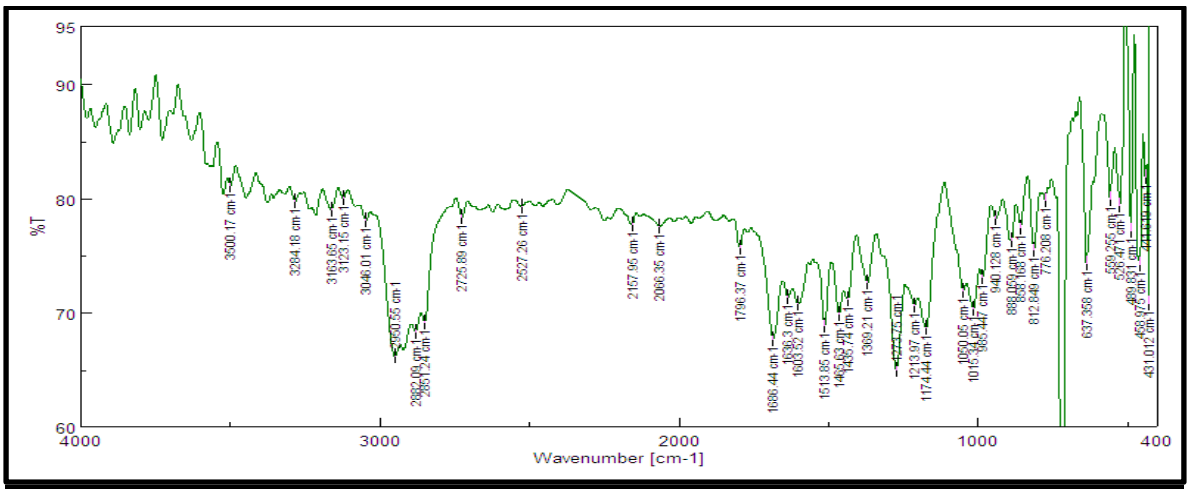
(ii)


100
95
90
85
%T
3696.87 cm-1
3504.99 cm-1
2919.7 cm-1
2850.27 cm-1
1733.69 cm-1
1174.44 cm-1
1089.58 cm-1
910.236 cm-1
643.144 cm-1
4000
3000
2000
1000
400
Wavenumber [cm-1]

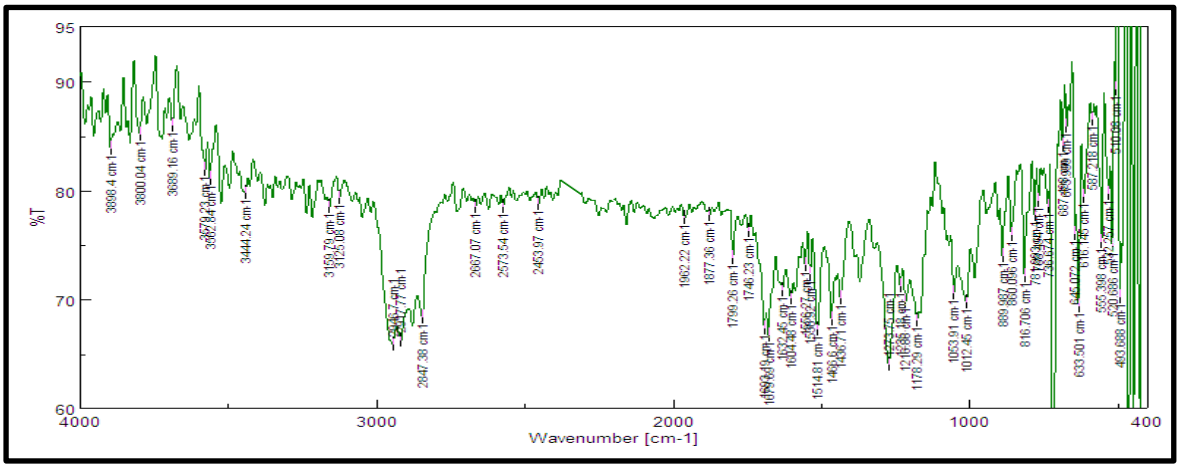
(iii)


110
100
80
60
40
%T
2955.39 cm-1
2846.35 cm-1
1662.34 cm-1
1468.53 cm-1
1211.08 cm-1
1083.8 cm-1
889.987 cm-1
816.706 cm-1
685.066 cm-1
637.368 cm-1
517.705 cm-1
4000
3000
2000
1000
400
Wavenumber [cm-1]

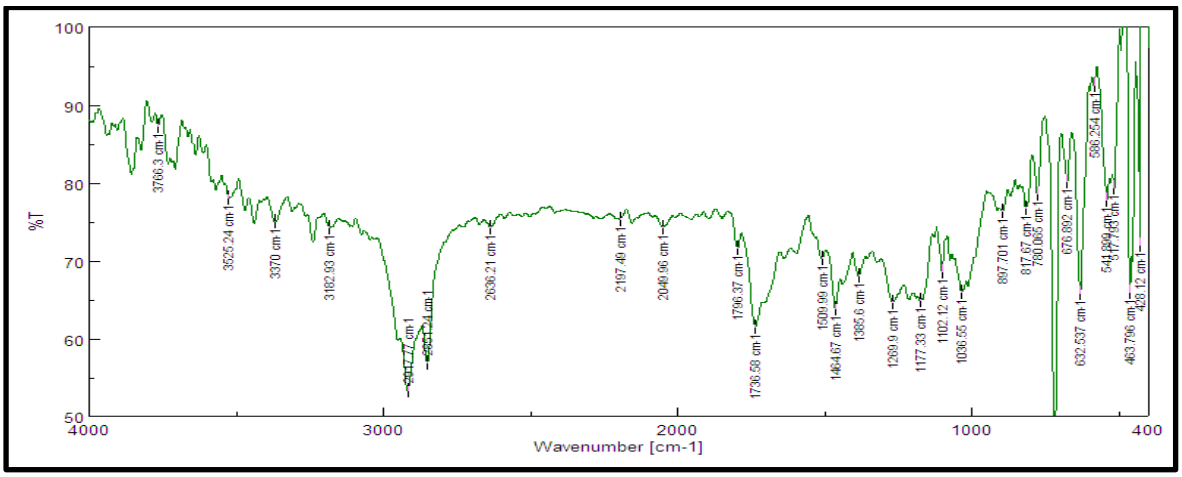
(iv)


95
90
80
70
60
%T
3500.17 cm-1
3284.18 cm-1
3163.65 cm-1
3123.15 cm-1
3046.01 cm-1
2950.55 cm-1
2882.09 cm-1
2851.24 cm-1
2725.89 cm-1
2527.26 cm-1
2157.95 cm-1
2066.35 cm-1
1796.37 cm-1
1686.44 cm-1
1636.3 cm-1
1603.52 cm-1
1513.85 cm-1
1465.63 cm-1
1438.74 cm-1
1369.21 cm-1
1274.76 cm-1
1213.97 cm-1
1174.44 cm-1
1050.05 cm-1
1015.39 cm-1
986.447 cm-1
940.128 cm-1
888.059 cm-1
868.168 cm-1
812.649 cm-1
776.208 cm-1
637.368 cm-1
559.255 cm-1
526.471 cm-1
489.831 cm-1
468.575 cm-1
431.012 cm-1
4000
3000
2000
1000
400
Wavenumber [cm-1]

(v)


%T
Wavenumber [cm-1]
4000
3000
2000
1000
400
95
90
80
70
60
3698.4 cm-1
3800.04 cm-1
3689.16 cm-1
3444.24 cm-1
3150.70 cm-1
3125.08 cm-1
2947.38 cm-1
2667.07 cm-1
2573.54 cm-1
2453.97 cm-1
1962.22 cm-1
1877.36 cm-1
1799.26 cm-1
1746.23 cm-1
1514.81 cm-1
1178.29 cm-1
1053.91 cm-1
1012.46 cm-1
889.667 cm-1
816.706 cm-1
633.501 cm-1
493.688 cm-1

(vi)


%T
Wavenumber [cm-1]
4000
3000
2000
1000
400
100
90
80
70
60
50
3766.3 cm-1
3525.24 cm-1
3370 cm-1
3182.93 cm-1
2947.77 cm-1
2636.21 cm-1
2197.49 cm-1
2049.96 cm-1
1736.58 cm-1
1509.99 cm-1
1464.67 cm-1
1395.6 cm-1
1269.9 cm-1
1177.33 cm-1
1102.12 cm-1
1036.55 cm-1
897.701 cm-1
817.67 cm-1
780.065 cm-1
676.892 cm-1
632.537 cm-1
598.264 cm-1
463.796 cm-1
428.12 cm-1

(vii)

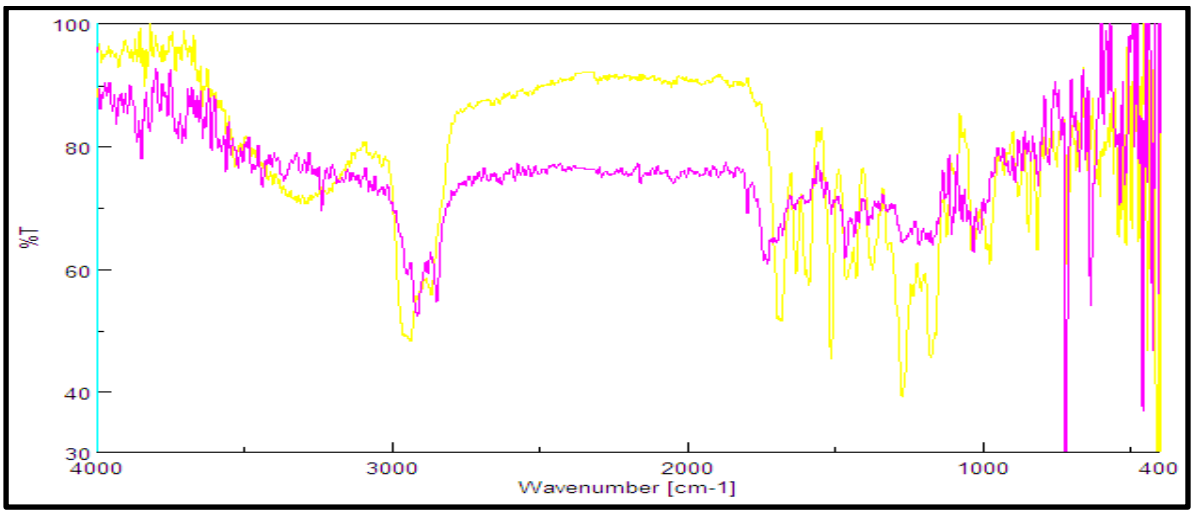
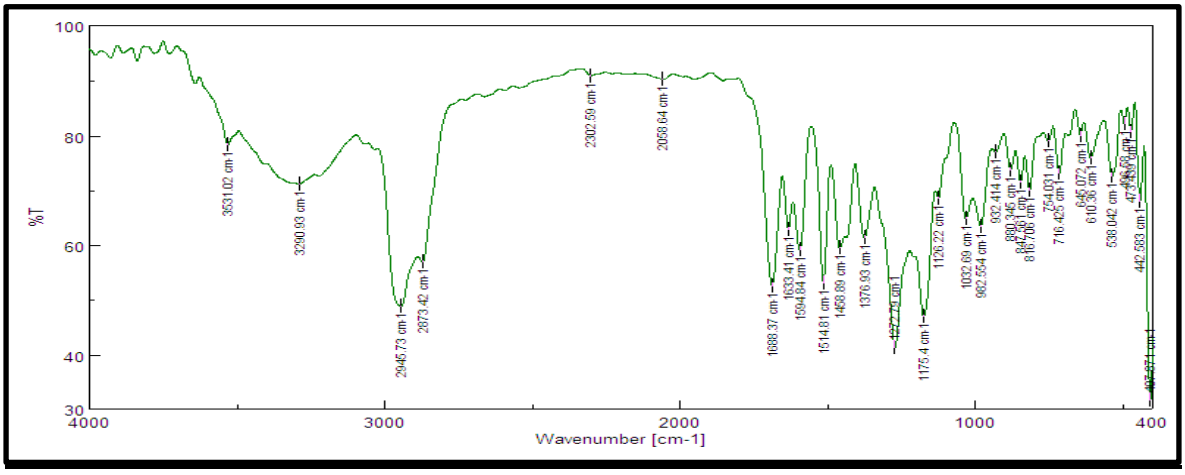

%T
Wavenumber [cm-1]
4000
3000
2000
1000
400
100
80
60
40
30

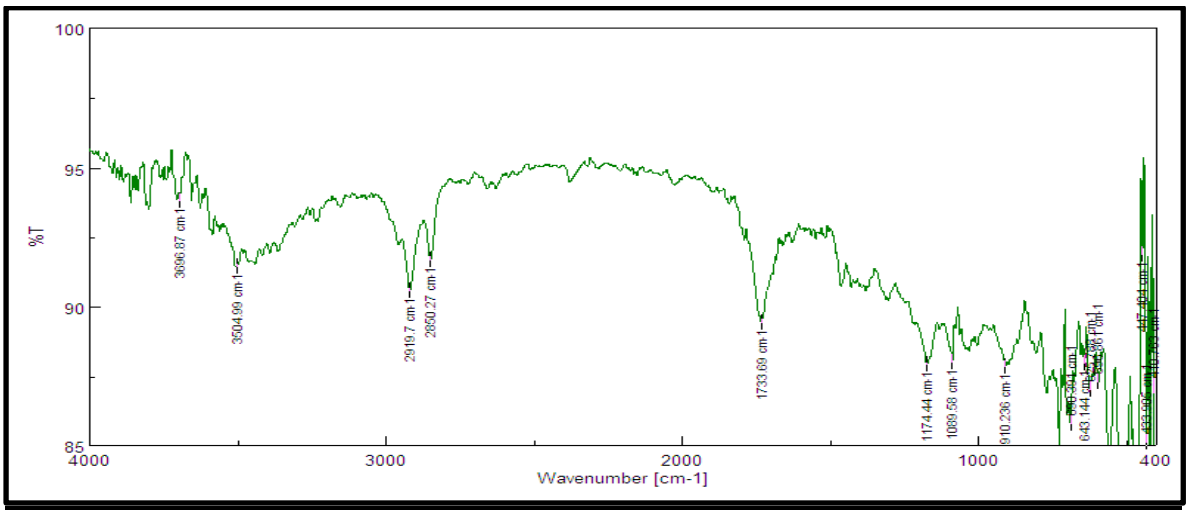
Fig4.7 IR Spectra of (i) drug OZ (OZ) (ii) span 60 (iii) Dicetly phosphate (DCP) (iv) OZ+ span 60 (v) OZ+ DCP (vi) OZ+ span 60 + DCP (vii) overlapped graph of OZ and OZ+ span 60 + DCP at initial time point

B) Observed IR spectra after 1 month

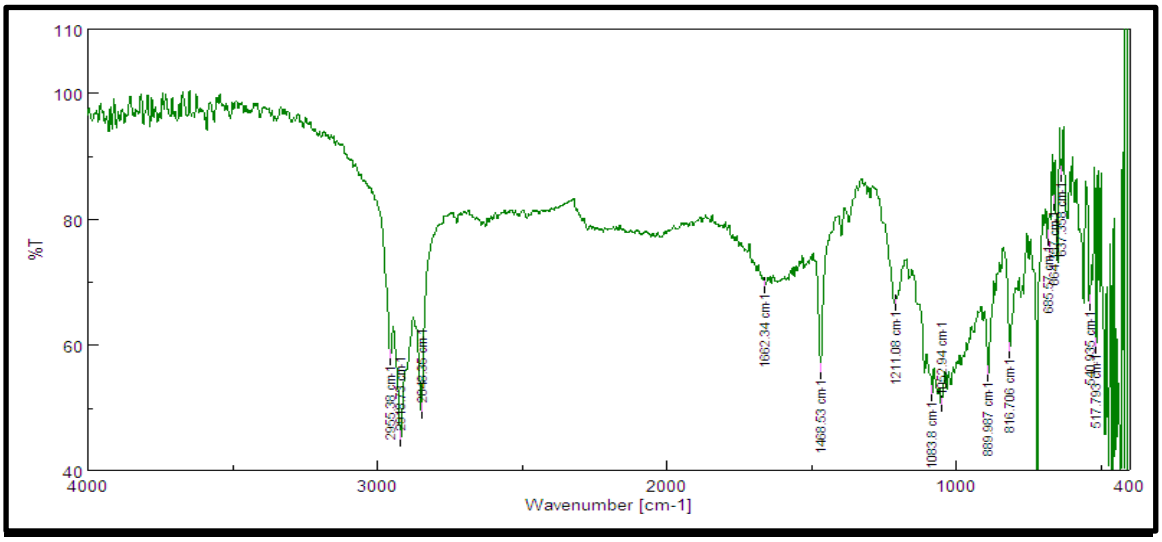
(i)



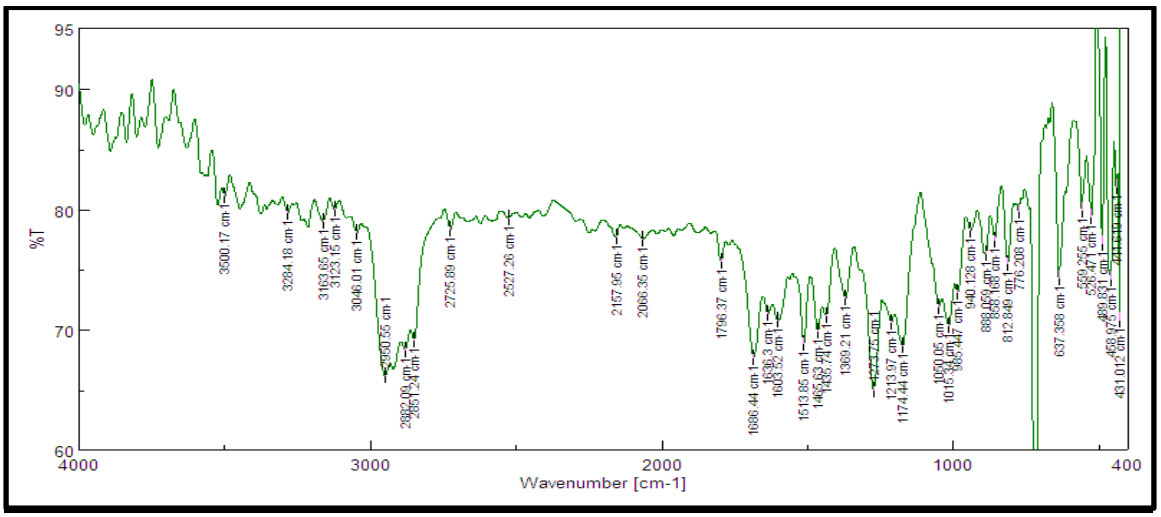
(ii)



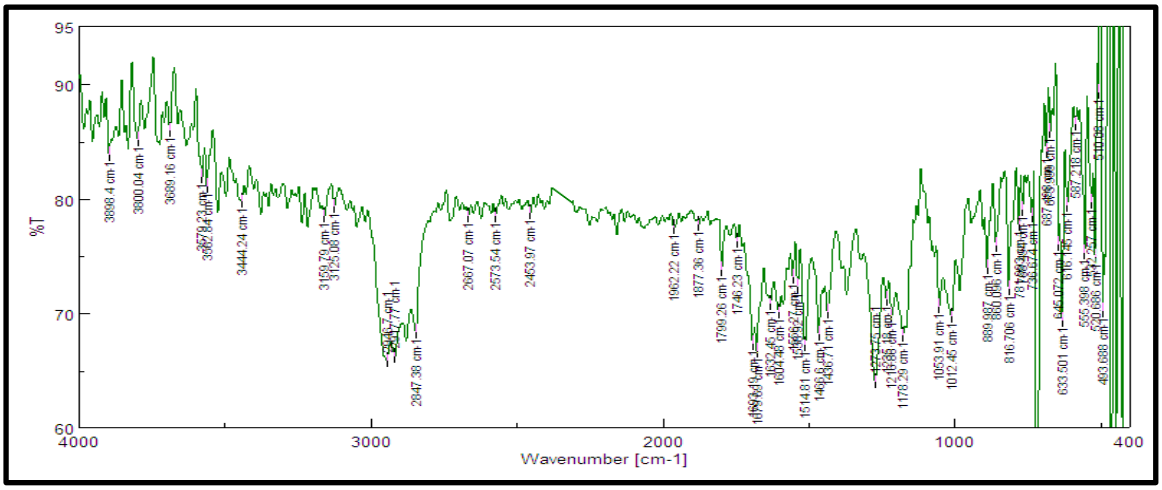
(iii)


%T
Wavenumber [cm-1]
4000
3000
2000
1000
400
110
100
80
60
40
2965.38 cm-1
2846.35 cm-1
1662.34 cm-1
1468.53 cm-1
1211.08 cm-1
1083.8 cm-1
1022.94 cm-1
889.987 cm-1
816.706 cm-1
685.56 cm-1
517.705 cm-1

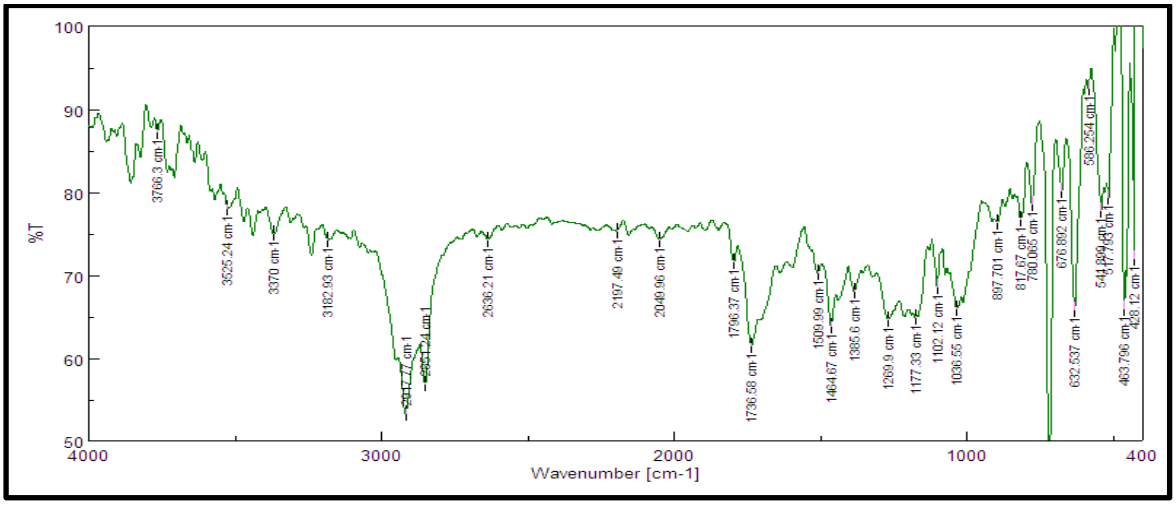
(iv)


%T
Wavenumber [cm-1]
4000
3000
2000
1000
400
95
90
80
70
60
3500.17 cm-1
3284.18 cm-1
3163.65 cm-1
3123.15 cm-1
3046.01 cm-1
2950.55 cm-1
2882.09 cm-1
2851.24 cm-1
2725.89 cm-1
2527.26 cm-1
2157.95 cm-1
2066.35 cm-1
1796.37 cm-1
1686.44 cm-1
1638.3 cm-1
1603.52 cm-1
1513.85 cm-1
1465.63 cm-1
1369.21 cm-1
1213.97 cm-1
1174.44 cm-1
1050.05 cm-1
1015.33 cm-1
965.447 cm-1
940.126 cm-1
889.069 cm-1
812.849 cm-1
776.208 cm-1
637.368 cm-1
559.255 cm-1
526.471 cm-1
489.831 cm-1
458.927 cm-1
431.012 cm-1

(v)


%T
Wavenumber [cm-1]
4000
3000
2000
1000
400
95
90
80
70
60
3898.4 cm-1
3800.04 cm-1
3689.16 cm-1
3444.24 cm-1
3159.79 cm-1
3125.08 cm-1
2847.38 cm-1
2667.07 cm-1
2573.54 cm-1
2463.97 cm-1
1962.22 cm-1
1877.36 cm-1
1799.26 cm-1
1746.23 cm-1
1514.81 cm-1
1178.29 cm-1
1053.91 cm-1
1012.45 cm-1
889.987 cm-1
816.706 cm-1
633.501 cm-1
555.396 cm-1
463.688 cm-1

(vi)



(vii)

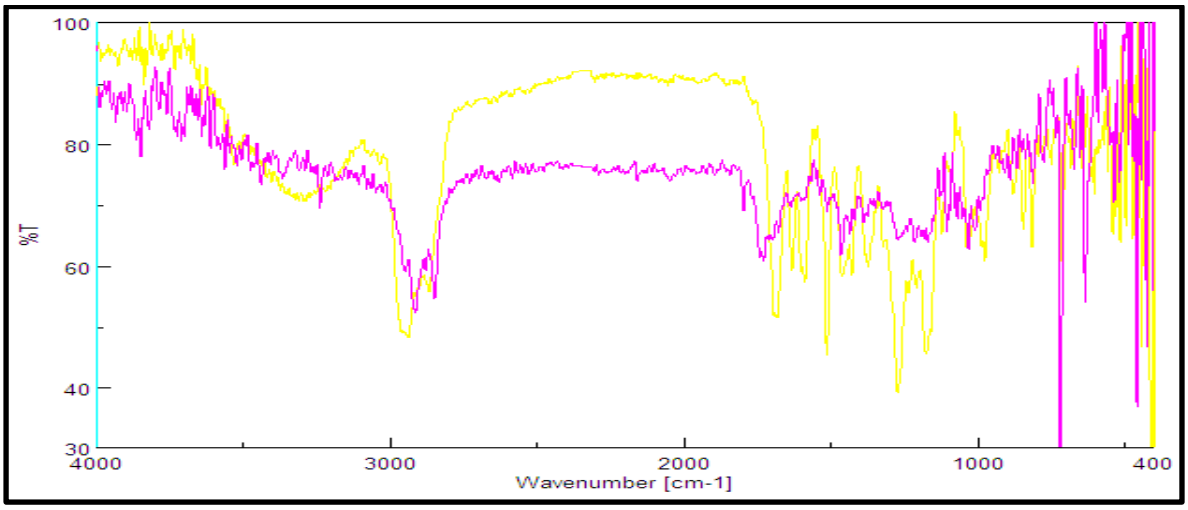


Fig 4.8: IR Spectra of (i) drug OZ (OZ) (ii) span 60 (iii) Dicetly Phosphate (DCP) (iv) OZ+ span 60 (v) OZ+ DCP (vi) OZ+ span 60 + DCP (vii) overlapped graph of OZ and OZ+ span 60 + DCP after 1 month

Table 4.11 Comparative IR frequencies of drug and drug with all selected excipients after one month

Functional groups	**OZ frequencies**	**OZ+span60+DCP frequencies**
Alkanes (C-H Stretching)	2945.73	2914.88
Aromatic hydrocarbon(C=C streching))	1633.41, 1458.89	1467.56
Alcohol (intermolecular H-bonded OH streching)	3269.93	3240.79
Ester(C=O streching)	1688.37	1729

Discussion: Compatibility studies for the drug and excipients were performed using FTIR spectrophotometer. OZ (OZ) and various excipients mixtures showed their respective characteristic peak bands & no change in peak observed in any of the samples of drug and excipients and combination of all by FT-IR. The drug and mixture of all excipients retained the entire characteristic peaks of OZ even after 1month. (Table: 4.11) Hence, drug was found to be compatible with all selected excipients**.**

4.5 Method of preparation of niosomes [49-53]

4.5.1 Solvent injection method:

This method is essentially based on slow injection of an organic solution of niosomal ingredients into an aqueous medium at high temperature. Due to higher temperature, this method cannot be used for thermo labile drugs. The solvents used for preparation may include diethyl ether, chloroform, Dichloromethane, Ethanol, etc. Niosomes in the form of large unilamellar vesicles (LUV) are formed by this method. The diameter of the vesicle ranges from 50 to 1000 nm depending on the conditions of the preparation. The flow chart of the preparation of niosomes by solvent injection method is shown in figure 4.9

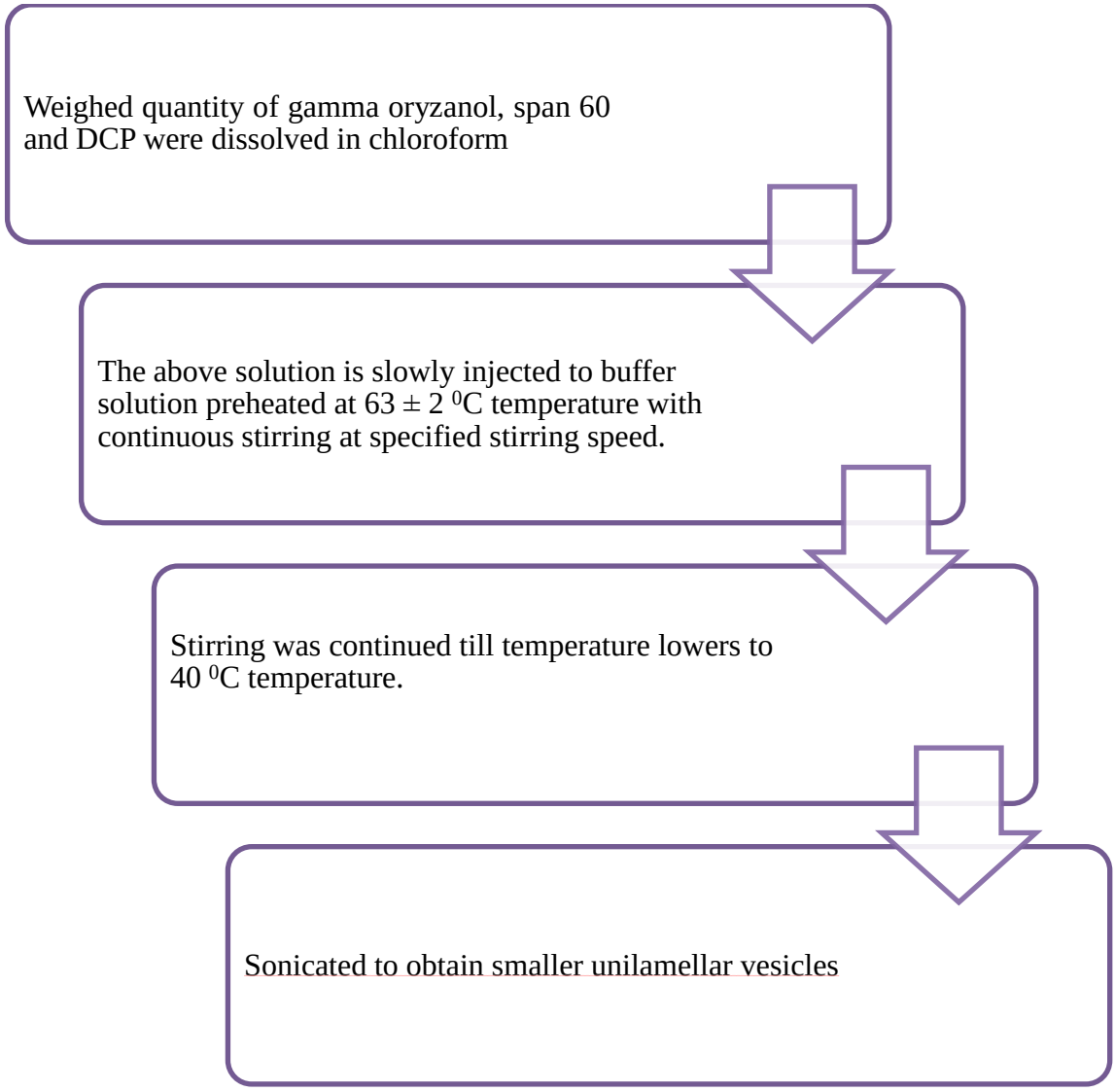


Figure 4.9 Flowchart describing steps for preparation of OZ loaded niosomes by solvent injection method

4.5.2 Thin film hydration method

Thin film hydration method is also known as hand shaking method. In this method the temperature used for evaporation of organic solvent is lower as room temperature not exceeding 40 ^{0}C. Due to lower temperature, this method is widely used for thermo labile drugs, proteins and peptides. This process yields typically multi lamellar vesicles. Lamellarity and size can further be reduced by high frequency probe sonication. Steps for preparation of niosomes by thin film hydration method is shown in figure 4.10

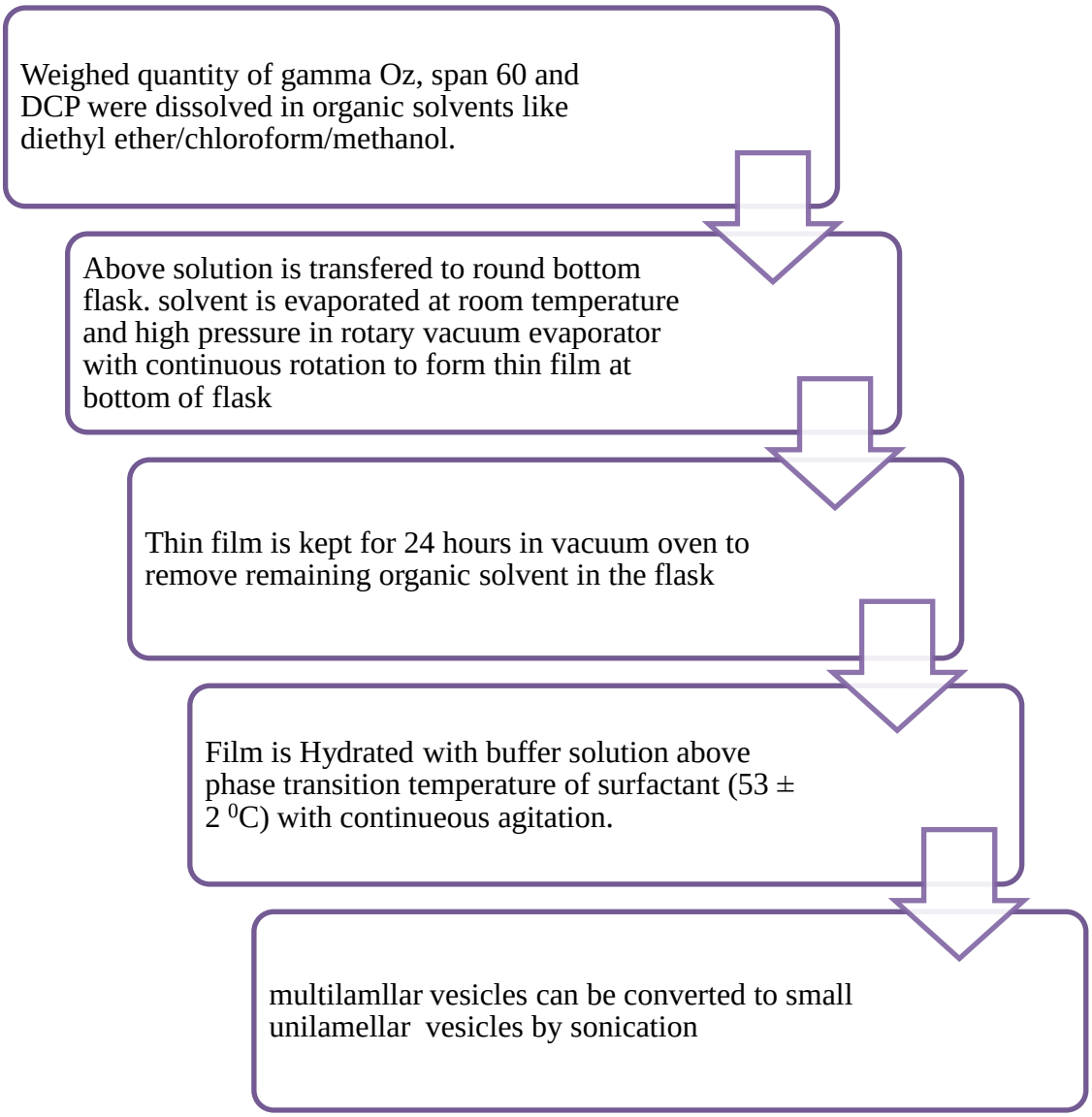


Figure 4.10Flowchart describing steps for preparation of OZ loaded niosomes by thin film hydration method

4.6 Characterization of niosomes [54-57]

4.6.1 Characterization of all batches of OZ loaded niosomes:

1) Average vesicle size and polydispersity index (PDI):

The average size of maximum population of niosomes was determined by DLS (dynamic light scattering) using Malvern zetasizer taking background of PBS 7.4. PDI (Polydispersity index) was also measured during the size measurement by same instrument. The graph of % intensity and size in nm was obtained.

2) % Drug entrapment efficiency (% EE):

It is the amount of drug entrapped in the niosomes. Entrapment efficiency was calculated by two methods. Before calculation of entrapment efficiency, % drug loading was calculated.

% Drug loading

- It is the total amount of drug present in niosomal solution = **Amount of drug added - drug loss.**
- The niosomal solution is diluted 100 times with ethanol and performed UV at 326 nm.
- The obtained concentration of drug was considered as total drug concentration for calculation of entrapment efficiency.

$$\textbf{\% EE} = \frac{\boldsymbol{total\ drug - freedrug}}{\boldsymbol{totaldrug}} \times \mathbf{100}$$

A. Centrifugation method

Samples were centrifuged at 15000 rpm (G=15120) for 15 minutes at 4 ^{0}C temperature by using Cooling centrifuge of REMI Motors (Optima L-100K Rotor). The supernatant was collected and diluted with phosphate buffer 7.4 to measure its absorbance at 327 nm in UV spectrophotometer. The supernatant gives amount of free drug. Hence by putting value to above equation EE can be calculated.

The drawback of centrifugation method is that it requires very high speed (i.e. G= 150000) for submicron particles, which could deform the vesicles and hence gives false results.

Equation for conversion of rpm to Force:

$$\mathbf{G\ (RCF) = 1.12 \times Radius \times (rpm/1000)^2}$$

B. Solid phase extraction method (SPE)

SPE is a process of separation of liquid sample on the basis of absorption principle. It consists of C-18 column and has tendency to retain non polar compound.

Niosomes can be eluted as first eluent with phosphate buffer 7.4 and diluted with ethanol. Ethanol used for dilution breaks niosomes and the absorbance is measured at 326 nm in UV spectrophotometer. The second eluent obtained with ethanol contains unentrapped drug. The % EE can be calculated by considering any of the concentration i.e. entrapped or unentrapped drug. The steps for obtaining % EE by SPE column is shown in figure 4.12

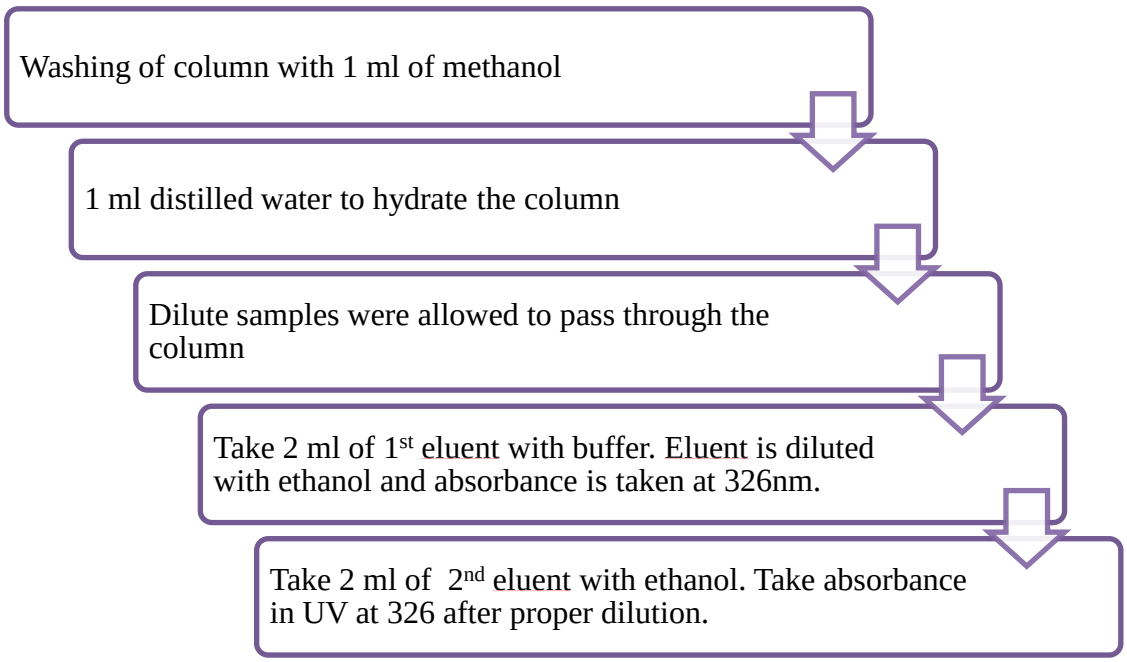


Figure 4.11 Steps for obtaining % EE by SPE

4.6.2 Characterization of optimized batch of gamma OZ loaded niosomes

1) Zeta potential (Surface Charge) measurement:

Zeta Potential represents the surface charge and stability of solution. Niosomal dispersions with Zeta Potential values greater than +30 mV or less than -30 mV generally have high degrees of stability and dispersions with a low zeta potential value will tend to aggregate due to Van der Waal interactions in between the vesicles. The zeta potential was measured using Malvern Zetasizer ZS200 at 25±0.5°C. All samples were measured in triplicate. The results are expressed as the mean voltage. Average size of optimized batch was also measured using Malvern Zetasizer.

2) Surface Morphology Study/Transmission Electron Microscopy:

The morphological characteristics of the OZ loaded niosomes were examined using a high resolution transmission electron microscope (TEM, Tecnai 20, Philips; Holland).A droplet of suspension was placed on a carbon film-covered copper grid (200 # mesh)without being stained. Five minutes later, the excess liquid was removed by touching the edge of the copper grid with a piece of filter paper. The sample was then air-dried before observation by TEM.

3) *Ex vivo* permeation studies:

The Ex vivo permeation of OZ from the OZ loaded Niosomes was studied by using skin of freshly sacrificed rat using **Franz diffusion cell** placed in a diffusion cell assembly. The diffusion medium used was freshly prepared PBS 7.4 solution. A rat skin was placed between the donor and receptor compartments of the diffusion cell. Three ml of formulation was accurately placed into this assembly. The receptor compartment has 20 ml of diffusion medium and the skin was kept such that it touches diffusion medium. The temperature was set at 37°C and rpm was kept at 100. Aliquots, each of 2 ml volume were withdrawn at regular intervals and replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with ethanol and their absorbance was determined by UV-Visible Spectrophotometry at 326 nm. Drug diffused after each time

points and the drug remained in donor compartment were calculated. From that the % drug retained in the skin was calculated.

% Drug retained =

$$\% \, \boldsymbol{of initial drug} - (\% \, \boldsymbol{drug diffused} + \% \, \boldsymbol{drug remained in donar compartment})$$

4) Stability studies:

Stability is defined as the extent to which a product remains within specified limits throughout its period of storage and use. It was carried out as per the regulatory guidelines at 25°C and 60% RH and refrigerated condition (4°C±2°C) in closed condition.

4.7 Method for preparation of niosomal Gel

Various polymers can be used for preparation of niosomal gel. Carbopol is widely accepted polymer which forms transparent and clear gel. It is a pH sensitive polymer, forms gel at or above neutral pH. The process flow for preparation of niosomal gel is shown in figure 4.12

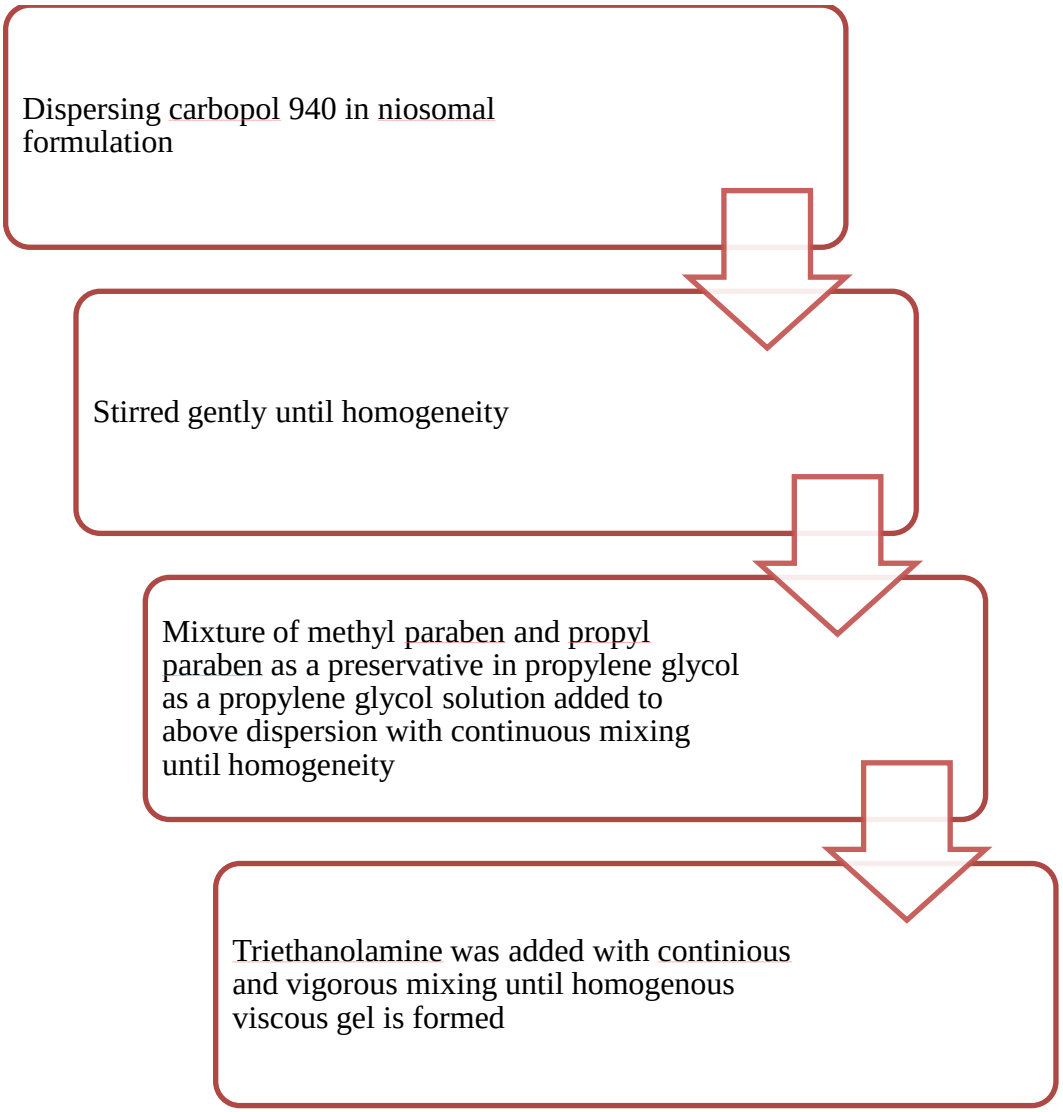


Figure 4.12 Process flow for preparation of niosomal gel

4.8 Characterization of niosomal Gel

1) pH Measurement:

The pH of gel has to be in the specified range of skin pH, hence pH of all formulations were measured by using digital pH meter by taking reference pH by buffer solution.

2) Viscosity: It is a measure of flow property. Brookfield viscometer was used to measure the viscosity of all batches. The spindle used was 18 at 0.3 rpm and the torque obtained was 85% or more in all batches.

3) Spreadability: The gel based system should be evaluated on the basis of its spreadability. The aqueous system tends to spread in larger area; hence its spreadability should be controlled for patient compliance. Glycerol or propylene glycols were added to improve spreadability and texture of gel. It can be checked by simply applying the fixed quantity of gel at the back of hand.

4) Draize test (Skin irritation study test on rabbits): It is an acute toxicity test; procedure involves applying of 0.5 ml of niosomal gel on the shaved and washed skin of conscious rabbit and leaving it for set amount of time before observing its effect. The rabbits were kept under normal room temperature with regular diet for 14 days. After 14 days the signs for any erythema, edema or redness were seen and recorded. For this test albino rabbits were used. After completion of test, if any serious injury or damage occurred to rabbit, euthanasia was provided otherwise animals were reused after wash out period.

5) *Ex vivo* permeation studies: The Ex vivo permeation of OZ from the OZ loaded niosomal gel was studied by using skin of freshly sacrificed rat using **Franz diffusion cell** placed in a diffusion cell assembly. The diffusion medium used was freshly prepared PBS 7.4 solution. A rat skin was placed between the donor and receptor compartments of the diffusion cell. One ml of formulation was accurately placed into this assembly. The receptor compartment has 20 ml of diffusion medium and the skin was kept such that it touches diffusion medium. The temperature was set at 37°C and rpm was kept at 100. Aliquots, each of 2 ml volume were withdrawn at regular intervals and replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with ethanol and

their absorbance was determined by UV-Visible Spectrophotometry at 326 nm. Drug diffused after each time points and the drug remained in donor compartment were calculated. From that the % drug retained in the skin was calculated.

% Drug retained =

$$\%\ \boldsymbol{ofinitialdrug} - (\%\ \boldsymbol{drugdiffused} + \%\ \boldsymbol{drugremainedindonarcompartment})$$

6) Stability studies:

Stability is defined as the extent to which a product remains within specified limits throughout its period of storage and use. It was carried out as per the regulatory guidelines at 25°C and 60% RH in closed condition.

4.9 Preliminary Trials:

4.9.1 Preliminary trials for selection of method of preparation for gamma OZ loaded niosomes

The two widely used methods for the preparation of niosomes are solvent injection method and thin film hydration method.

4.9.1.1 Preparation of blank niosomes by solvent injection and thin film hydration method without using DCP (Charge inducer)

Blank niosomes without using charge inducer were prepared; diethyl ether was selected as a solvent for injection. While in thin film hydration method, Chloroform: methanol (2:1 v/v) was taken as solvent system. Different molar ratios of Span 60 to cholesterol (1:0.5; 1:1; 1:1.5; 1:2) were taken. Distilled water was taken as media for hydration. Process parameters for ether injection method were kept constant shown in table below. The composition of all the batches and their evaluation were shown in tables below

Table 4.12 Constant values of process parameters

Process Parameters	**Constant values**
Temperature for ether injection method (^{0}C)	60
Stirring speed (rpm)	1000
Flow rate of injection (ml/min)	0.5
Temperature for TFH method (^{0}C)	40
Rotation speed for TFH method (rpm)	100

Table 4.13 Composition of blank niosomes by solvent injection method

Solvent injection method				
Ingredients	**E1**	**E2**	**E3**	**E4**
Span 60	43.6 (mg)	43.6 (mg)	43.6 (mg)	43.6 (mg)
Cholesterol (Molar ratio)	19.3 (mg) (1:0.5)	38.6 (mg) (1:1)	57.9 (mg) (1:1.5)	73.2 (mg) (1:2)
Diethyl ether	5 ml	5 ml	5ml	5ml
Distilled water	20 ml	20ml	20ml	20ml

Table 4.14 Composition of blank niosomes by Thin Film hydration method

Thin Film hydration method				
Ingredients	**F1**	**F2**	**F3**	**F4**
Span 60	43.6 (mg)	43.6 (mg)	43.6 (mg)	43.6 (mg)
Cholesterol(Molar ratio)	19.3 (mg) (1:0.5)	38.6 (mg) (1:1)	57.9 (mg) (1:1.5)	73.2 (mg) (1:2)
Chloroform: methanol (2:1 v/v)	13 ml	13 ml	13 ml	13 ml
Distilled water	10 ml	10ml	10ml	10ml

Evaluation

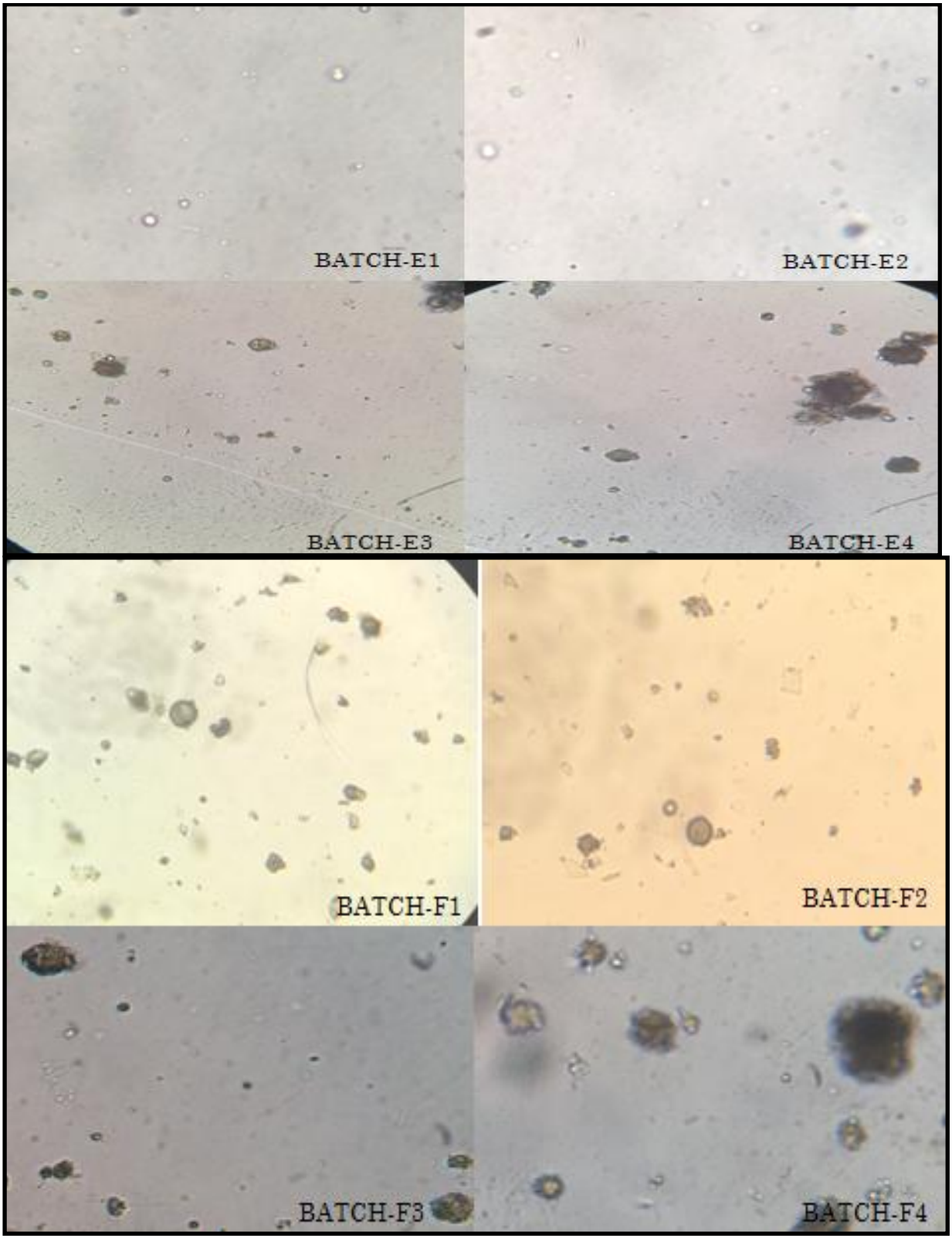


Figure 4.13 Microscopic images of all batches taken in inverted optical microscope at 45x power

Observations

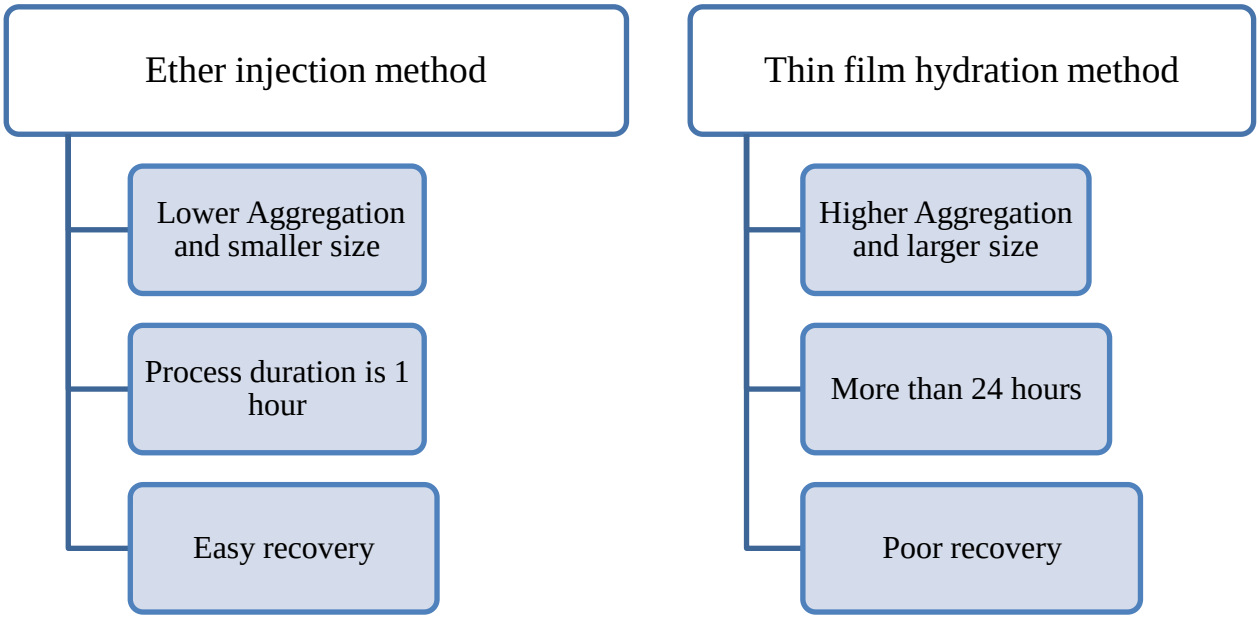


Figure 4.14 Observations obtained from preliminary trails of blank niosomes

4.9.1.2 Preparation of drug loaded niosomes by above method without DCP

Trial batches were prepared by above two methods with drug loading in niosomes. The ratio of span 60: cholesterol was kept constant at 1: 0.5, to compare the drug loading between two methods. Other parameters were kept same as previous batches. Composition of batches prepared and their evaluation parameters are mentioned in tables below (4.15 & 4.16).

Table 4.15 Composition of drug loaded batch by solvent injection method and evaluation

Ingredients	E1D	% Drug Entrapped
Drug (mg)	25	51.40 %
Span 60 (mg)	43.6	
Cholesterol (mg) Ratio	19.3 (1:0.5)	
Diethyl ether	5 ml	
Distilled water	20 ml	

Table 4.16 Composition of drug loaded batch by thin film hydration method and evaluation

Ingredients	F1D	% Drug Entrapped
Drug	25 (mg)	45.87 %
Span 60	43.6 (mg)	
Cholesterol	19.3 (mg) (1:0.5)	
Chloroform: methanol (2:1 v/v)	13 ml	
Distilled water	10 ml	

Result: The entrapment efficiency of both formulations were obtained, which showed that the solvent injection method has higher drug entrapped in niosomes than with Thin Film hydration method.

Discussion: Solvent injection method showed better recovery, smaller size, and lower aggregation than in Thin Film hydration method, hence former can be used for optimization of formulation.

4.9.2 Preliminary trials for the screening of important factors affecting gamma OZ niosomes prepared by solvent injection method

Gamma Oz niosomes were prepared by solvent injection method and various formulation and process parameters were studied to optimize them to get final formulation having smaller vesicular size and higher entrapment of drug. On the basis of literature review a systematic risk assessment is performed to identify CMAs and CPP whose change may influence potential CQAs. The relative risk that components present was ranked as high, medium, or low. Those attributes that could have a high impact on the formulation CQAs required further investigation whereas those attributes that had low impact on the formulation CQAs required no further investigation. The relative risk ranking system was summarized in Table 4.17 and some initial risk analysis was done, which is shown in table 4.17 (a).The justification for the risk prioritization is presented in Table 4.17 (b). Each formulation component that has a high risk to impact the CQAs is further evaluated

in subsequent risk assessments to determine which formulation variables need to be studied to reduce the risk.

Table 4.17 Overview of relative risk ranking system

Low	Broadly acceptable risk. No further investigation is needed
Medium	Risk is accepted. Further investigation may be needed in order to reduce the risk
High	Risk is unacceptable. Further investigation is needed to reduce the risk

Table 4.17 (a) Risk analysis

Process and formulation variables									
CQAs	**Temp**	**Flow rate**	**Span-60: Cholesterol ratio**	**Charge Inducer (DCP)conc.**	**Needle size**	**Sonication**	**Volume of hydration**	**Drug: span-60 ratio**	**Stirring speed**
Vesicle Size	High	medium	High	Medium	Medium	High	High	Medium	High
% EE	Low	Low	High	Low	Low	High	Medium	High	High
Zeta potential	low	Low	Low	High	Low	Low	Low	Low	Low

Table 4.17(b) Justification of risk prioritization

Components/ Variables	**Formulation CQAs**	**Justification**
Temperature	Vesicle Size	As the temperature of hydration was reduced below phase transition temperature of span-60 it forms lumps hence risk of impact on vesicle size is high. It requires further evaluation
	% EE	The risk of temperature to impact entrapment efficiency is low.
	Zeta potential	The risk of temperature to impact zeta potential is low.
Flow rate	Vesicle Size	The flow rate of injection can influence the vesicle size; increase in the flow rate may reduce vesicle size hence it is under medium risk.
	% EE	The injection flow rate has low impact on entrapment efficiency. Hence do not require further investigation.
	Zeta potential	The risk of flow rateto impact zeta potential is low.
Span-60: cholesterol ratio	Vesicle Size	The ratio of span-60 to cholesterol affects the vesicle strength. Hence it affects the vesicle size.
	% EE	As the drug is hydrophobic, it entrap within the bilayer, same as cholesterol. Hence ratio of span-60 to cholesterol is considered under high risk level.
	Zeta potential	The risk of span-60 to cholesterol ratio to impact zeta potential is low.

Charge inducer (DCP) concentration	Vesicle Size	DCP is a negative charge inducer; it has a indirect effect on vesicle size. As the DCP concentration increases negative charge increases which induce repulsive force between vesicles. Hence decrease aggregation and vesicle size. So it is considered under medium risk
	% EE	The risk of DCP comcentration on entrapment efficiency is under low impact.
	Zeta potential	Zeta potential is highly affected by concentration of charge inducer. Hence it is under high risk and requires further investigation.
Needle size	Vesicle Size	The needle size can affect the vesicle size, as the size of droplet increases the chances the vesicle size may increase. Hence it is under medium risk which requires further investigation
	% EE	The impact of needle size on entrapment efficiency is low. It do not require any further investigation
	Zeta potential	The impact of needle size on zeta potential is low. It do not require any further investigation
Sonication	Vesicle Size	Sonication affects the vesicle size. With increase in Sonication the vesicle size decreases. Hence it is under high risk
	% EE	Sonication may break vesicles which may reduces entrapment efficiency. Hence it si under high risk.
	Zeta potential	The risk of sonication on zeta potential is under low impact.

Volume of hydration	Vesicle Size	The volume of hydration increases the size of vesicle linearly and hence it requires further investigation.
	% EE	The impact of volume of hydration on entrapment efficiency is medium. Because at lower volume of hydration vesicle may bear high shear force which may reduce EE.
	Zeta potential	The risk of volume of hydration on zeta potential is under low impact.
Drug: span-60 ratio	Vesicle Size	As drug is lipophilic it gets entrapped in bilayer of vesicle, hence it may affect the vesicle size. Hence it is under medium risk.
	% EE	The ratio of drug to span-60 has higher impact on entrapment efficiency. Hence it require further in
	Zeta potential	The risk of Drug: span-60 ratio to impact zeta potential is low.
Stirring speed	Vesicle Size	Stirring speed affects the vesicle size directly. Hence it is under higher risk.
	% EE	Stirring speed gives shear to vesicle which may break vesicles and hence reduce entrapment efficiency.
	Zeta potential	The risk of stirring speed to impact zeta potential is low.

4.9.2.1 Effect of temperature

Gamma OZ niosomes were prepared by solvent injection method by varying one of the process parameter (Temperature). Temperature of the aqueous hydration media is an important parameter to be optimized. The phase transition temperature of span 60 is 52 ± 3 ^{0}C, hence two batches with three different temperatures (50, 60, 70 ^{0}C) were tried. The composition of batches and evaluation is as shown in below tables (4.18 & 4.19)

Table 4.18 Composition of batches for optimization of temperature

Ingredients	Z1	Z2
Drug	25 mg	25 mg
Span 60	43.6 mg	87.2 mg
Cholesterol	19.3 mg	19.3 mg
DCP	8 mg	8 mg
Chloroform	5 ml	5 ml
PBS 7.4	10 ml	10 ml
Flow rate	0.5 ml/min	0.5 ml/min
Stirring speed	1000 rpm	1000 rpm
Needle size	18 G	18 G

Evaluation

Table 4.19 Evaluation of batches for optimization of temperature

Batch	Temperature (^{0}C)	Appearance
Z1	50	Lumps and aggregates
Z2	50	Lumps and aggregates
Z1	60	Lesser Aggregates
Z2	60	Lesser aggregates
Z1	70	More lumps
Z2	70	More lumps

Result: Z1 and Z2 batches showed lesser aggregates at 60 ^{0}C temperature; which is above phase transition temperature of span 60. Hence it is necessary to keep temperature above 55 ^{0}C.

Discussion: Both the batches Z1 and Z2 showed similar results at same temperature, which proves that temperature has effect on the size. Also the formation of lumps was may be due to unentrapped drug; hence temperature also affects entrapment efficiency. From the above results it shows that the temperature should be in the range of 60 – 65 ^{0}C. At higher temperature (70 ^{0}C) more lumps were seen. Also at temperature above 70 ^{0}C drug is not stable.

4.9.2.2 Effect of injection flow rate

Optimization of flow rate was done in this step by keeping all other parameters constant. Composition of batches by varying flow rate of injection and their evaluation parameters are shown in below tables (4.20 & 4.21)

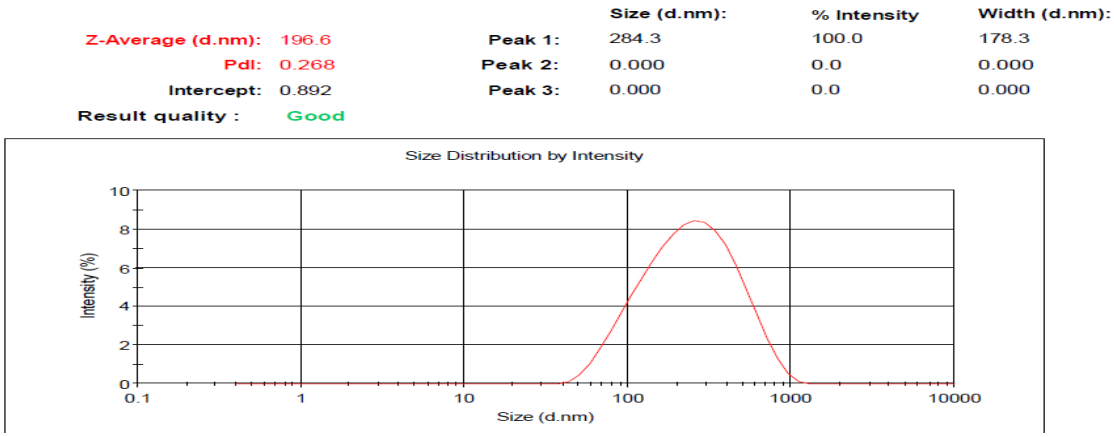
Table 4.20 Composition of batches for optimization of flow rate

Ingredients	Batch
Drug	25 mg
Span 60	43.6 mg
Cholesterol	19.3 mg
DCP	8 mg
Chloroform	5 ml
PBS 7.4	10 ml
Stirring speed	1000 rpm
Needle size	18 G
Temperature	60 ± 3^{0}C

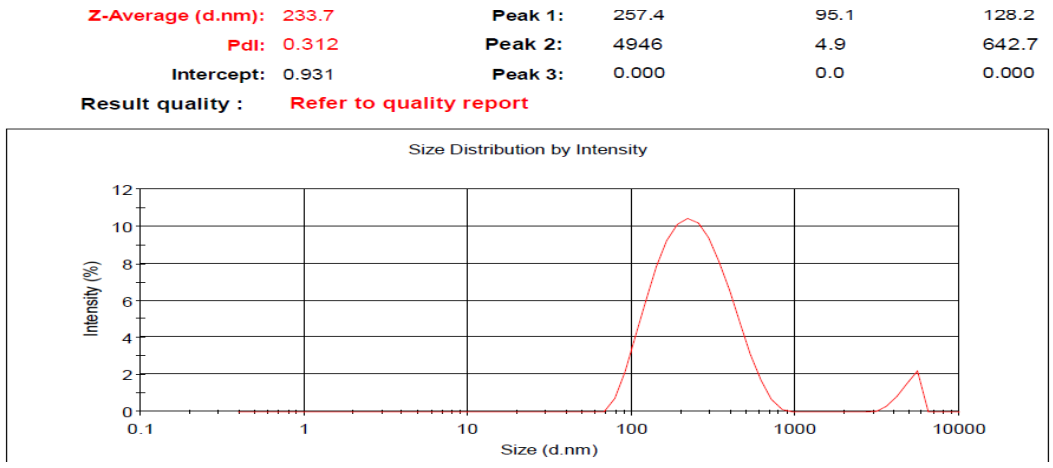
Evaluation

Table 4.21 Evaluation of batches for optimization of flow rate

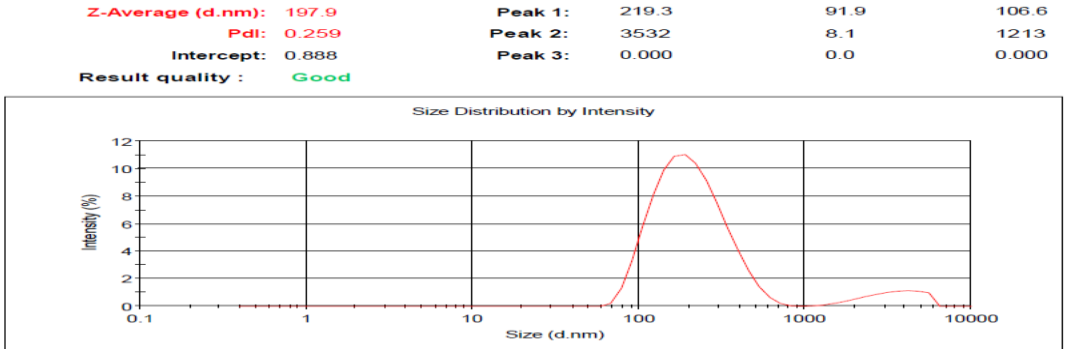
Batch	FR1	FR2	FR3	FR4
Flow rate (ml/min)	0.5	1	1.5	2.0
Average Vesicle size	196	233	197	207
Polydispersity index (PDI)	0.268	0.312	0.259	0.264



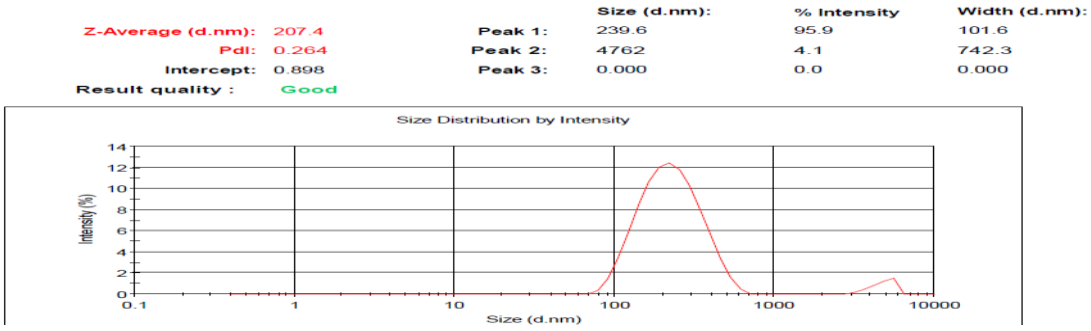
FR1



FR2



FR3



FR4

Figure 4.15 Average Vesicle size of batches FR1 – FR4

Result and Discussion: The average vesicle size and PDI showed non linear response with flow rate of injection. Hence change in flow rate does not affect vesicle size and PDI significantly. Hence for reducing the time for the formulation process higher flow rate of 2 ml/min can be used.

4.9.2.3 Effect of span-60: cholesterol ratio

Optimization of span cholesterol ratio was done in this step by keeping all other parameters constant. Composition of batches by varying span cholesterol ratio and their evaluation parameters are shown in below tables (4.22 & 4.23)

Table 4.22 Composition of batches for optimization of span-60: cholesterol ratio

Batch	Drug (mg)	DCP (mg)	Temperature (^{0}C)	Stirring speed (rpm)	Chloroform (ml)	PBS 7.4 (ml)	Flow rate (ml/ min)	Span 60 : Cholesterol ratio (molar w/w)
A1	50	8	60 ± 3	1000	5	100	2	2:2
A2	50	8	60 ± 3	1000	5	100	2	2:1
A3	50	8	60 ± 3	1000	5	100	2	3:1
A4	50	8	60 ± 3	1000	5	100	2	3:0.5
A5	50	8	60 ± 3	1000	5	100	2	3:0

Evaluation

Table 4.23 Evaluation of batches for optimization of span-60: cholesterol ratio

Batch	Appearance	Entrapment efficiency %
A1	Lumps and Aggregation	35
A2	Lumps and Aggregation	37.50
A3	Aggregation	38.25
A4	Lower Aggregation	45.15
A5	No aggregation	48.21

Result and Discussion: As the concentration of cholesterol decreased, aggregation decreased this shows decrease in average vesicle size and hence stability of niosomal formulation. Also concentration of cholesterol affects the entrapment efficiency; it gives maximum entrapment efficiency and least aggregation without using cholesterol.

4.9.2.4 Effect of DCP concentration on formulation:

Optimization of DCP was done in this step by keeping all other parameters constant. Composition of batches by varying DCP concentration and their evaluation parameters are shown in below tables (4.24, 4.25& 4.25 (a)).

Table 4.24 Composition of batches for optimization of DCP concentration

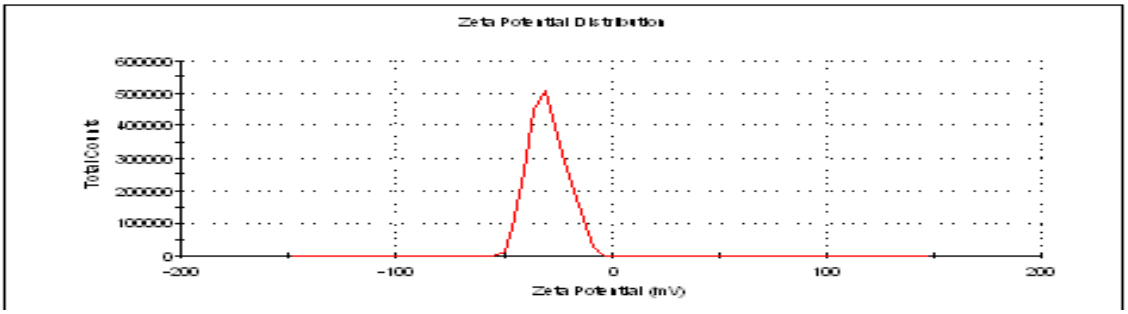
Ingredients	Batch
Drug	30.29 mg
Span 60	130.8 mg
Chloroform	5 ml
PBS 7.4	100 ml
Flow rate	2 ml/min
Stirring speed	1000 rpm
Needle size	18 G
Temperature	60 ± 3 (^{0}C)

Evaluation

Table 4.25 Evaluation of batches for optimization of DCP concentration

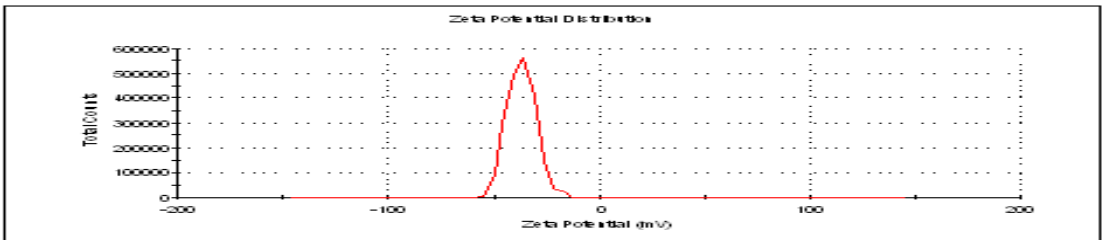
Batch	B1	B2	B3
DCP Conc. (mg)	8	16	24
Zeta potential (mV)	-29.5	-36.9	-41.6
Average vesicle size	214	188.9	178.45

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -29.5	Peak 1:	-29.5	100.0	8.64
Zeta Deviation (mV): 8.64	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0610	Peak 3:	0.00	0.0	0.00
Result quality : Good				



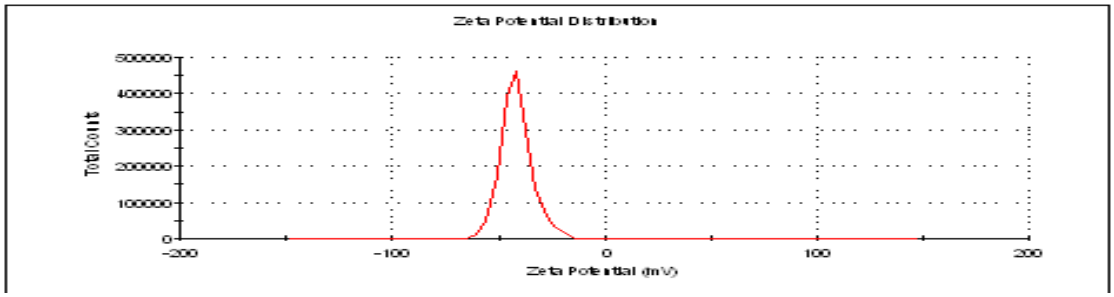
B1

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -36.9	Peak 1:	-36.9	100.0	6.76
Zeta Deviation (mV): 6.76	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0639	Peak 3:	0.00	0.0	0.00
Result quality : Good				



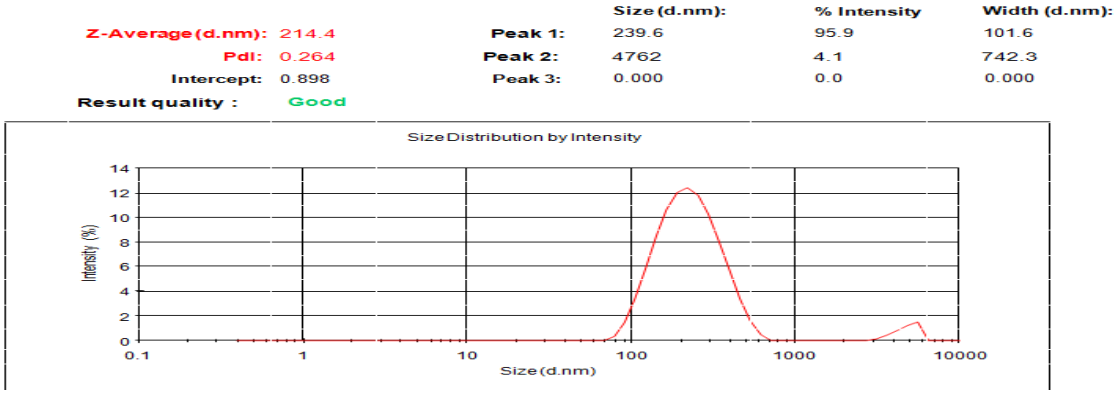
B2

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -41.6	Peak 1:	-41.6	100.0	7.28
Zeta Deviation (mV): 7.28	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0653	Peak 3:	0.00	0.0	0.00
Result quality : Good				

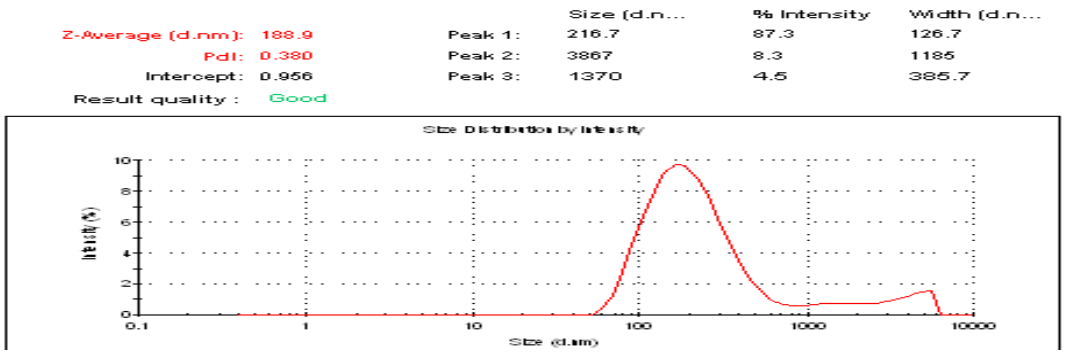


B3

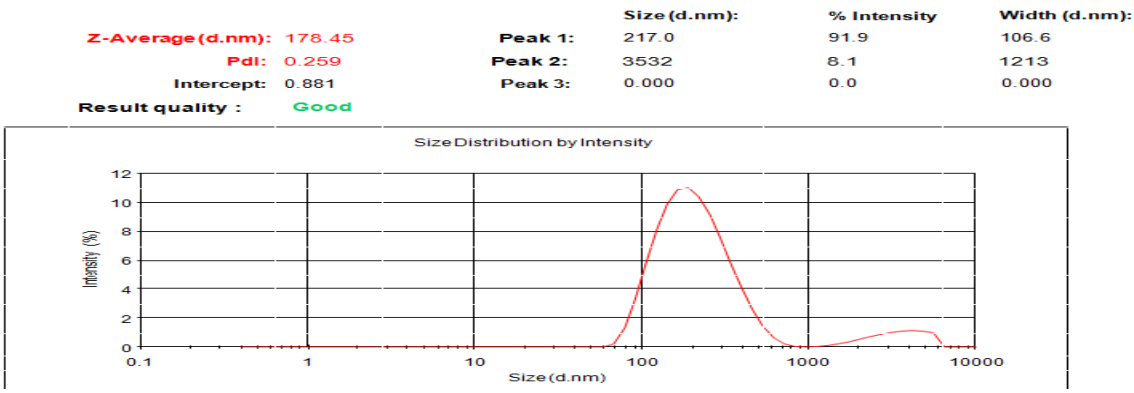
Figure 4.16 Zetta potential of batches B1 – B3



B1



B2



B3

Figure 4.16 (a) Average vesicle size of batches B1 – B3

Result and Discussion: It was observed that as the concentration of DCP increases it reduces the aggregation. This is because of the repulsive force developed by negative chargeon niosomes induced by DCP. Hence DCP plays an important role in stability of niosomes.The results of zeta potential shows that at the concentration of 8 mg and above it attains -30 mv charge which is under the criteria for stable formulation. Hence for further formulation optimization, DCP concentration can be kept between 8 mg to 16 mg.

Also the vesicle size decreased comparatively, because of repulsion, which do not allow formation of aggregates.

4.9.2.5 Effect of needle size:

Optimization of needle size was done in this step by keeping all other parameters constant except the Sonication. Here Sonication was not performed. As Sonication may reduce the vesicle size, it would be difficult to study the effect of needle size. Composition of batches and their evaluation parameters are shown in below tables (4.26 & 4.27)

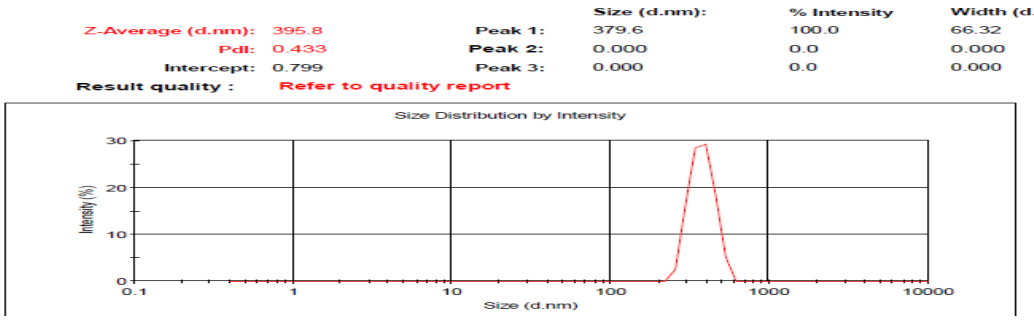
Table 4.26 Composition of batches for optimization of needle size

Ingredients	Batch
Drug	30.29 mg
Span 60	130.8 mg
DCP	8 mg
Chloroform	5 ml
PBS 7.4	100 ml
Flow rate	2 ml/min
Stirring speed	1000 rpm
Temperature	60 ± 3 (^{0}C)

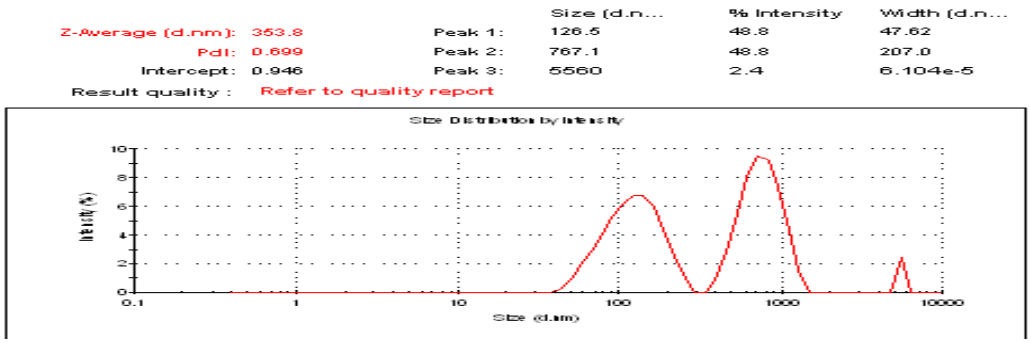
Evaluation

Table 4.27 Evaluation of batches for optimization of needle size

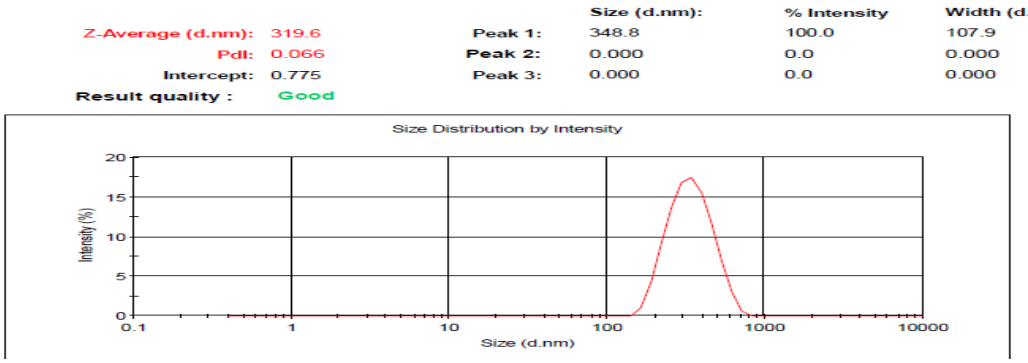
Batch	N1	N2	N3
Needle size (G)	18	22	**24**
Average Vesicle size (nm)	395.8	353.8	**319.6**
Polydispersity index	0.433	0.699	**0.066**



N1



N2



N3

Figure 4.17 Average vesicle size of batches N1 – N3

Result and Discussion: From the above observation it can be concluded that there is some effect of higher gauge size needleon Vesicle size and polydispersity index.Due to smaller vesicle size and lower PDI when compared with other two sizes, for further optimization 24 gauge needle can be used.

4.9.2.6 Effect of sonication:

Optimization of Sonication and Sonication time was done in this step at two different ratio of drug: span-60 by keeping all other parameters constant. Composition of batches and their evaluation parameters are shown in below tables (4.28 & 4.29)

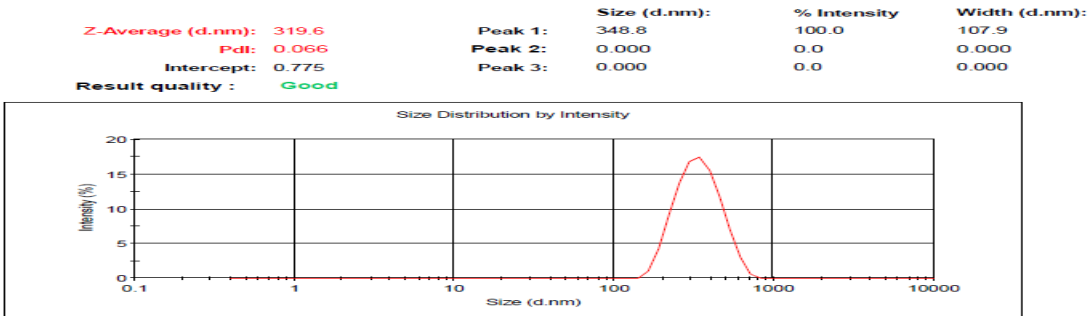
Table 4.28 Composition of batches for optimization of sonication and sonication time

Batch	Drug (mg)	Span 60 (mg)	DCP (mg)	Temperature (^{0}C)	Stirring speed (rpm)	Chloroform (ml)	PBS 7.4 (ml)	Flow rate (ml/ min)	Needle size (gauge)
S1	30.29	130.8	8	60 ± 3	1000	5	100	2	24
S2	60.58	130.8	8	60 ± 3	1000	5	100	2	24

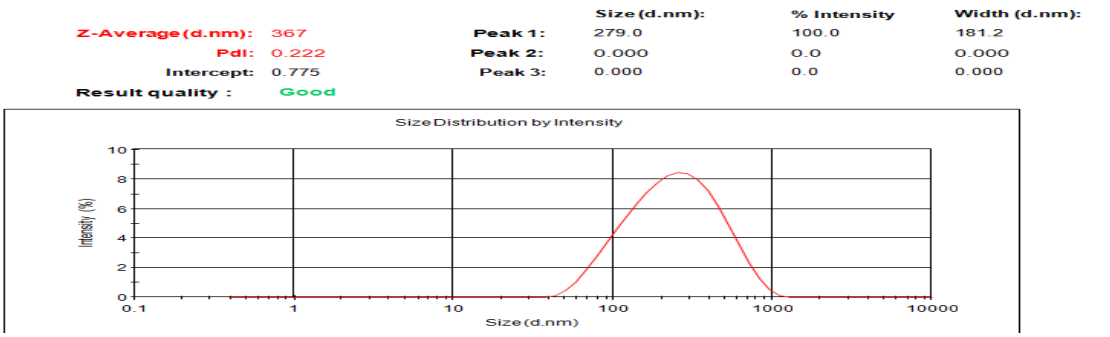
Evaluation

Table 4.29 Evaluation of batches for optimization of sonication and sonication time

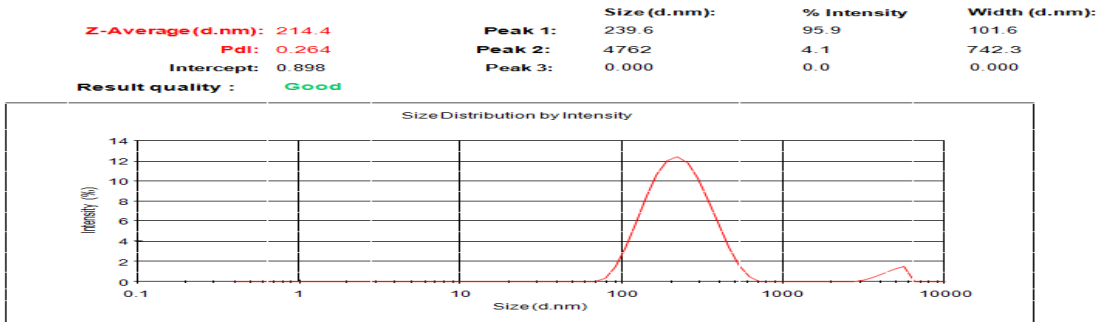
Batch	S1	S2
Average Vesicle size before sonication (nm)	319.6	367
Average Vesicle size after 5 minutes sonication (nm)	214	346.2
Entrapment efficiency before sonication (%)	67.50 %	72.41 %
Entrapment efficiency after 5 minutes sonication (%)	66.12 %	70.00 %
Entrapment efficiency after 15 minutes sonication (%)	42.01 %	46 %



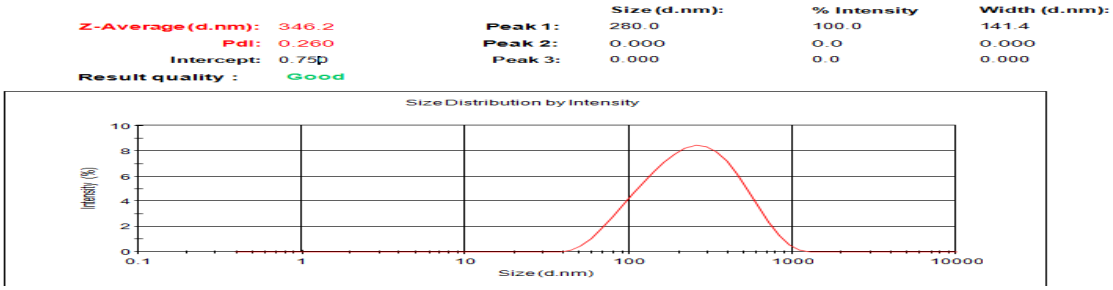
S1: Average vesicle size before sonication



S2: Average vesicle size before sonication



S1: Average vesicle size after 5 minutes sonication



S2: Average vesicle size after 5 minutes sonication

Figure 4.18 Average vesicle size of batches S1 and S2 (before and after sonication)

Result and Discussion: The average vesicle size in both the batches were decreased after sonication. But with increase in the sonication time, there was decrease in entrapment efficiency too. This was might be due to breaking of niosomes upon sonication for longer duration. Hence the sonication time should be kept at 5 minutes.

4.9.2.9 Effect of volume of hydration:

Optimization of volume of hydration was done in this step by keeping all other parameters constant. Composition of batches and their evaluation parameters are shown in below tables (4.30 & 4.31)

Table 4.30 Composition of batches for optimization of volume of hydration

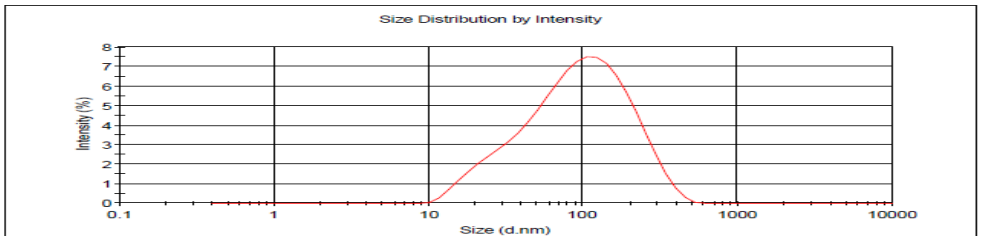
Ingredients	Batch
Drug	30.29 mg
Span 60	130.8 mg
DCP	8 mg
Chloroform	5 ml
Flow rate	2 ml/min
Stirring speed	1000 rpm
Temperature	60 ± 3 (^{0}C)
Needle size	24 G

Evaluation

Table 4.31 Evaluation of batches for optimization of volume of hydration

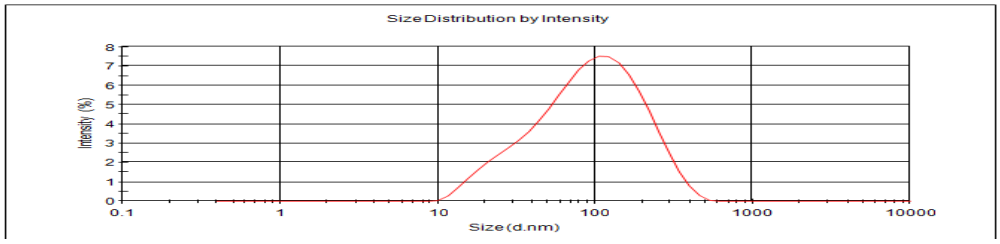
Batch	V1	V2	V3	V4
Volume of hydration (ml)	25	50	75	100
Average Vesicle size (nm)	67.65	96.5	178.45	214
Polydispersity index	0.41	0.21	0.25	0.26
Entrapment efficiency (%)	49.20	68.56	64.10	66.12

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	67.65	Peak 1:	113.8	100.0	81.37
PdI:	0.410	Peak 2:	0.000	0.0	0.000
Intercept:	0.671	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



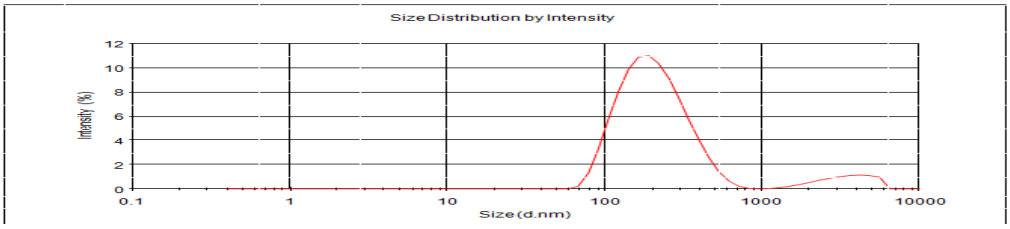
V1

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	96.5	Peak 1:	121.1	100.0	93.32
PdI:	0.210	Peak 2:	0.000	0.0	0.000
Intercept:	0.652	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



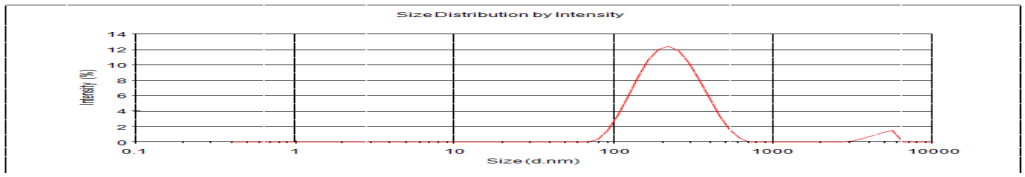
V2

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	178.45	Peak 1:	217.0	91.9	106.6
PdI:	0.259	Peak 2:	3532	8.1	1213
Intercept:	0.881	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



V3

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	214.4	Peak 1:	239.6	95.9	101.6
PdI:	0.264	Peak 2:	4762	4.1	742.3
Intercept:	0.898	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



V4

Figure 4.19 Average vesicle size of batches V1 – V4

Result and discussion: Volume of hydration showed linear response with average vesicle size. This may be due to the more aqueous media available for the vesicle to assemble. Hence the vesicle will entrap more amount of buffer and result in to larger size. While in case of entrapment efficiency there is not much effect of volume of hydration. Batch V1 showed low EE than all other batches, this may be due to lower volume; the impact of stirring could be higher which may reduce the entrapment of drug. As it is affecting both the parameters it needs further optimization.

4.9.2.10 Effect of drug: span-60 ratio:

Optimization of drug: span-60 ratio was done in this step by keeping all other parameters constant. Composition of batches and their evaluation parameters are shown in below tables (4.32 & 4.33)

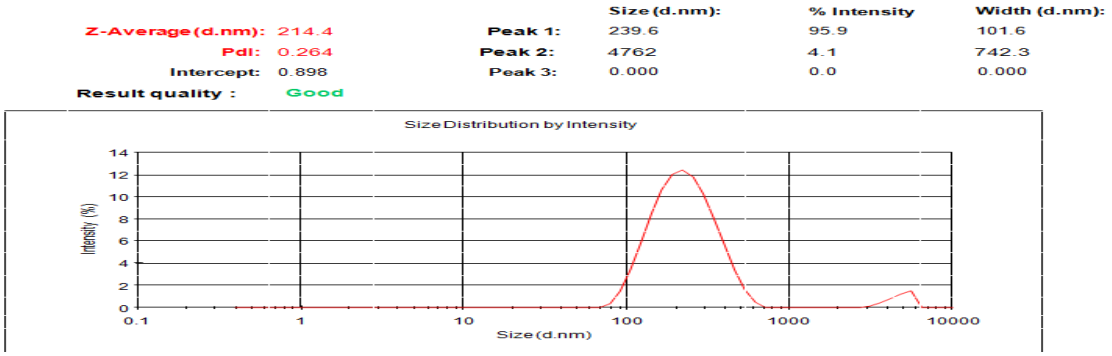
Table 4.32Composition of batches for optimization of drug: span-60 ratio

Ingredients	Batch
DCP	8 mg
Chloroform	5 ml
Volume of hydration	100 ml
Flow rate	2 ml/min
Stirring speed	1000 rpm
Temperature	60 ± 3 (^{0}C)
Needle size	24 G

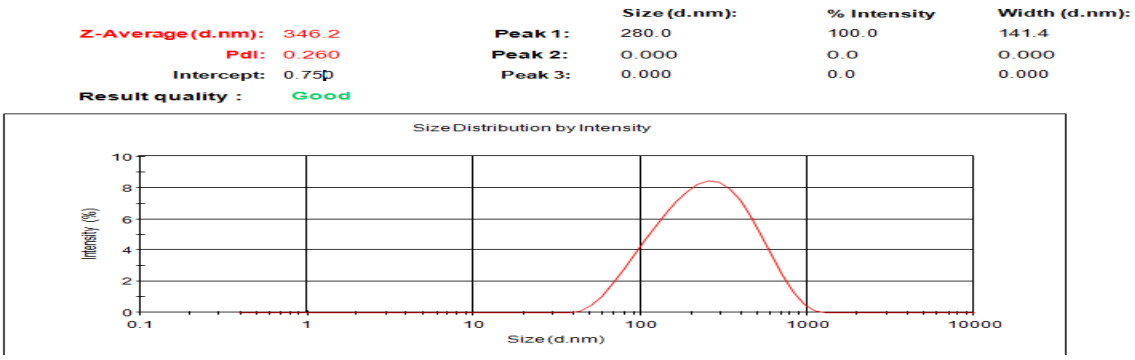
Evaluation

Table 4.33Evaluation of batches for optimization of drug: span-60 ratio

Batch	C1	C2
Drug (mg)	30.29	60.58
Span-60 (mg)	130.80	130.80
Average Vesicle size (nm)	214	346.2
Polydispersity index	0.264	0.260
Entrapment efficiency (%)	66.12	70.00



C1



C2

Figure 4.20 Average vesicle size of batches C1 nd C2

Result and discussion: Drug: span-60 ratio is having a high effect on particle size as well as on entrapment efficiency. Hence it requires further optimization.

4.9.2.11 Effect of stirring speed

Optimization of Drug: span-60 ratio was done in this step by keeping all other parameters constant. Composition of batches and their evaluation parameters are shown in below tables (4.34& 4.35)

Table 4.34 Composition of batches for optimization of drug: span-60 ratio

Ingredients	Batch
Drug	25.0 mg
Span-60	43.6 mg
Cholesterol	19.3 mg
DCP	8 mg
Chloroform	5 ml
Volume of hydration	100 ml
Flow rate	2 ml/min
Temperature	60 ± 3 (^{0}C)
Needle size	18 G

Evaluation

Table 4.35 Evaluation of batches for optimization of stirring speed

Batch	**H1**	**H2**	**H3**	**H4**
Stirring speed	800	1000	1200	1500
Appearance	Lumps were seen	Aggregation	Lower aggregation	No aggregation
Entrapment efficiency (%)	41.30	43.08	48.50	52.60

Result and Discussion: The effect of stirring speed was observed on formulation appearance and entrapment efficiency . It was found that there was decrease in the aggregation with increase in stirring speed. The reduction of aggregation shows that stirring speed is affecting particle size as well as PDI, this was may be due to increase in shear force with increase in stirring speed. It also affects the entrapment efficiency, with decrease in aggregation the amount of niosomes passing through SPE column increases which could be the reason of increase in entrapment efficiency. Hence stirring speed could be further optimized for getting maximum entrapment efficiency and smaller vesicle size.

Conclusion derived from the preliminary trials

As per the initial risk assesment, all the CMAs and CPPs were studied in preliminary trails to obtain desiredlevel of CQAs. Hence from the preliminary trails below mentioned factors were identified at lower risk and were kept constant for further study. The critical parameters are further considered for optimization by using 2^3 Full factorial design.

- Temperature: 62 ± 3 ^{0}C
- Flow rate: 2 ml/min
- DCP concentration: 8 mg
- Needle size: 24 gauge
- Sonication time: 5 minutes

Critical parameters needs optimization

- Drug: Span 60 ratio
- Volume of hydration
- Stirring speed

4.10 Optimization of OZ niosomes using 2^3 full factorial design

4.10.1 Full factorial design (2^3) for optimization of OZ loaded niosomes

Based on the preliminary trials, three parameters i.e. drug: span 60 ratios, volume of hydration, stirring speed were identified as critical parameters. To optimize these factors at two levels, 2^3 full factorial design was selected. Batches were prepared and evaluated.

4.10.2 Composition & design matrix of 2^3 full factorial design:

Table 4.36 Levels of variables in 2^3 full factorial design

Independent variables		
Factors	**Levels, Coded (Actual)**	
	Low	**High**
A: Drug: Span 60 molar ratio	-1 (1:4)	+1 (1:8)
B: Volume of hydration (PBS 7.4)	-1 (10)	+1 (100)
C: Stirring speed	-1 (1000)	+1 (2500)
Dependent variables		
R1: Average Vesicle size (nm) **R2:** Entrapment efficiency (%)		

Table 4.37 2^3full factorial design matrixes

Batch no.	Independent variables in coded form		
	A	B	C
D1	-1	-1	-1
D2	1	-1	-1
D3	-1	1	-1
D4	1	1	-1
D5	-1	-1	1
D6	1	-1	1
D7	-1	1	1
D8	1	1	1
D9	0	0	0
D10	0	0	0
D11	0	0	0

4.10.3 Response R1 (vesicle size distribution)

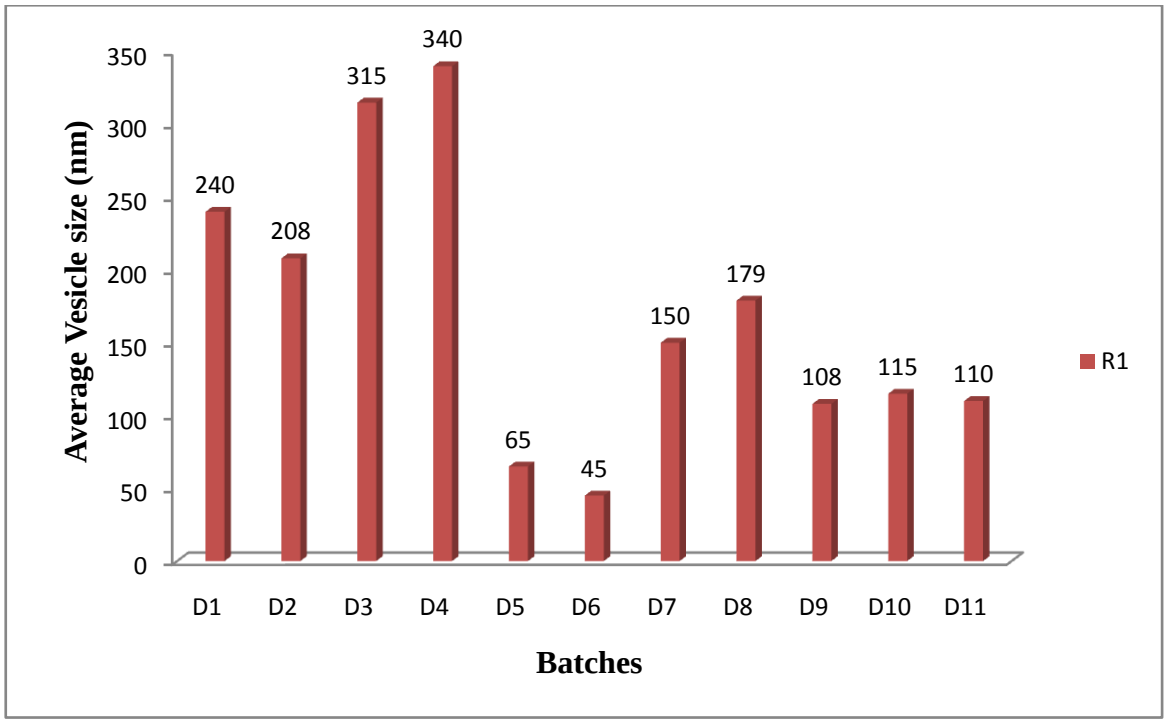
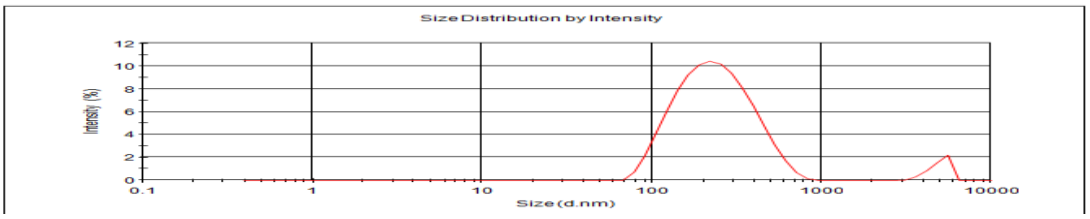


Figure 4.21Comparative chart of average vesicle size of factorial batches

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 240.6	Peak 1:	254.0	97.8	131.1
PdI: 0.210	Peak 2:	5120	4.6	630.7
Intercept: 0.831	Peak 3:	0.000	0.0	0.000

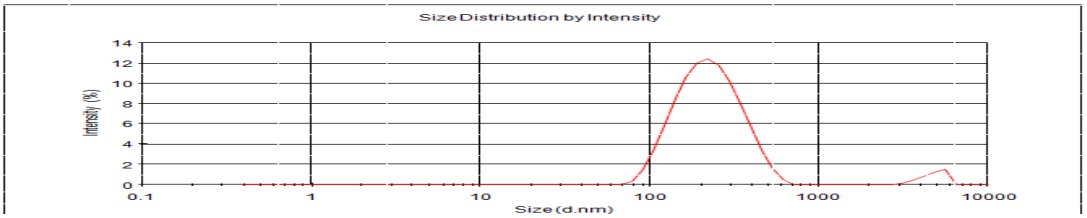
Result quality: Refer to quality report



D1

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 208.4	Peak 1:	239.6	95.9	101.6
PdI: 0.264	Peak 2:	4762	4.1	742.3
Intercept: 0.898	Peak 3:	0.000	0.0	0.000

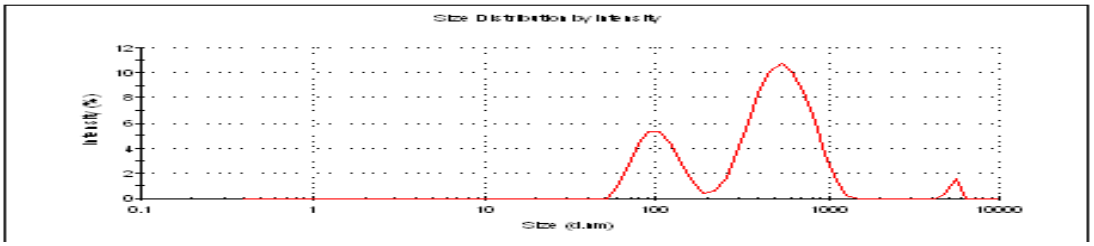
Result quality : Good



D2

		Size (d.n...	% Intensity	Width (d.n...
Z-Average (d.nm): 314.7	Peak 1:	558.9	71.0	200.9
PdI: 0.519	Peak 2:	103.9	27.1	28.64
Intercept: 0.956	Peak 3:	5430	1.9	285.2

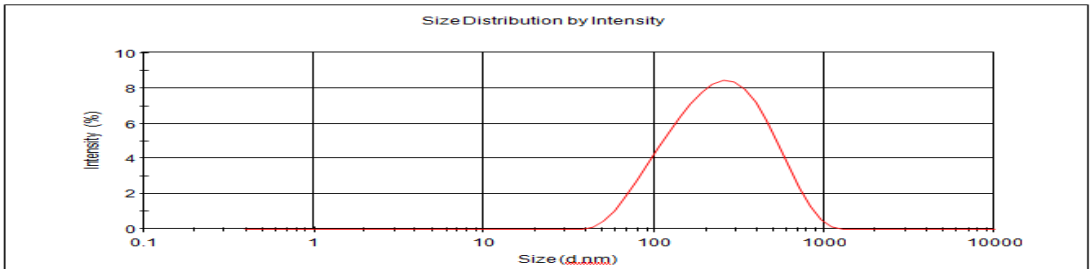
Result quality : Refer to quality report



D3

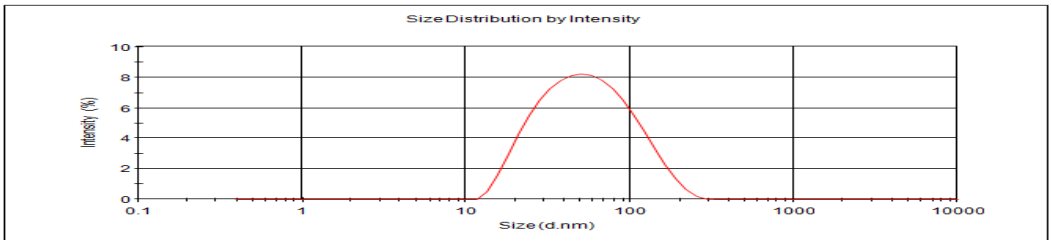
		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 340.2	Peak 1:	280.0	100.0	141.4
PdI: 0.260	Peak 2:	0.000	0.0	0.000
Intercept: 0.750	Peak 3:	0.000	0.0	0.000

Result quality : Good



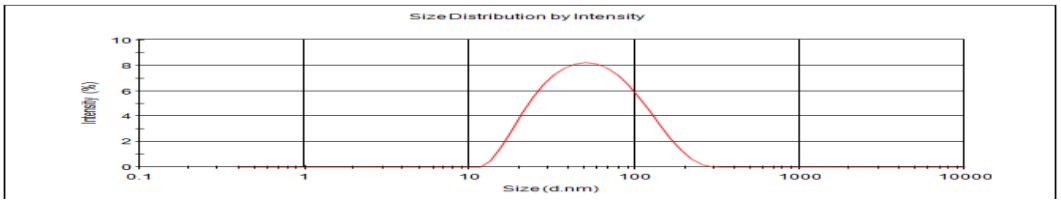
D4

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	65.12	Peak 1:	82.10	100.0	64.10
PdI:	0.222	Peak 2:	0.000	0.0	0.000
Intercept:	0.710	Peak 3:	0.000	0.0	0.000
Result quality:	Good				



D5

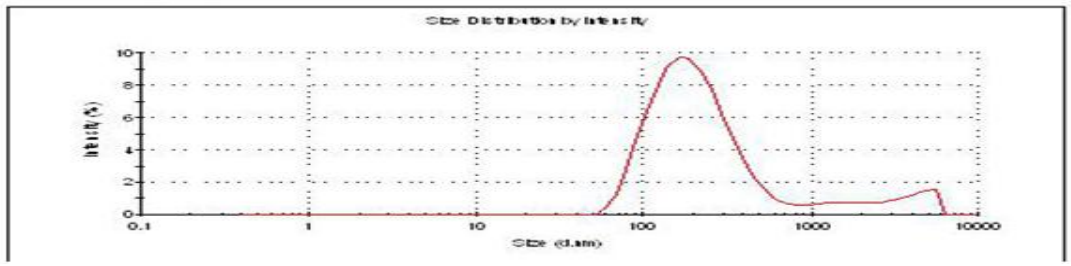
			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	45.16	Peak 1:	64.08	100.0	41.79
PdI:	0.241	Peak 2:	0.000	0.0	0.000
Intercept:	0.656	Peak 3:	0.000	0.0	0.000
Result quality:	Good				



D6

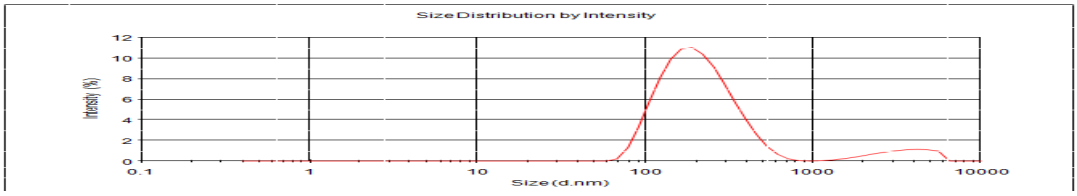
			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	150.4	Peak 1:	257.4	95.1	128.2
PdI:	0.312	Peak 2:	4946	4.9	642.7
Intercept:	0.931	Peak 3:	0.000	0.0	0.000

Result quality: Good



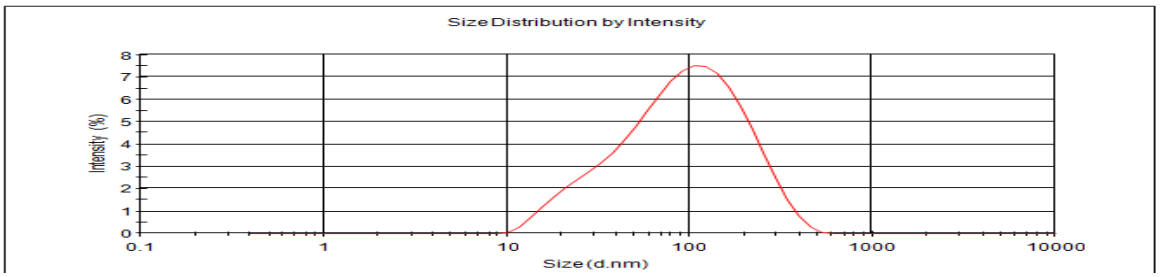
D7

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	178.45	Peak 1:	217.0	91.9	106.6
PdI:	0.259	Peak 2:	3532	8.1	1213
Intercept:	0.881	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



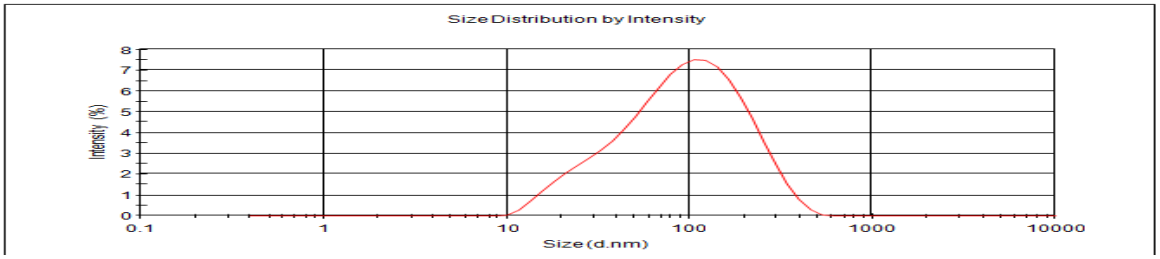
D8

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 107.9	Peak 1:	121.1	100.0	93.32
PdI: 0.210	Peak 2:	0.000	0.0	0.000
Intercept: 0.652	Peak 3:	0.000	0.0	0.000
Result quality: Good				



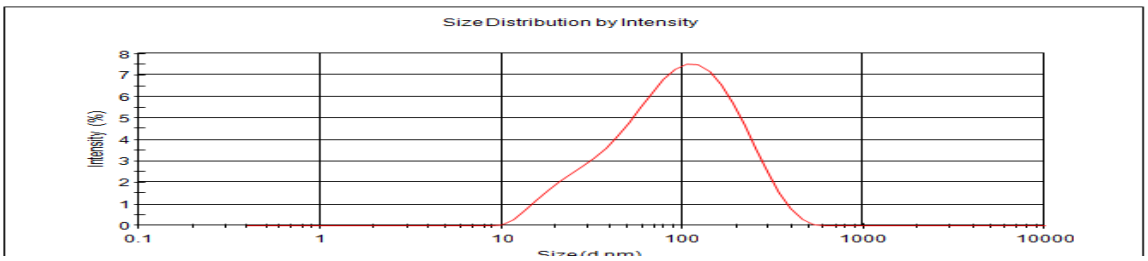
D9

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 115.0	Peak 1:	116.8	100.0	96.71
PdI: 0.312	Peak 2:	0.000	0.0	0.000
Intercept: 0.712	Peak 3:	0.000	0.0	0.000
Result quality: Good				



D10

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 110.5	Peak 1:	150.3	100.0	80.92
PdI: 0.279	Peak 2:	0.000	0.0	0.000
Intercept: 0.640	Peak 3:	0.000	0.0	0.000
Result quality: Good				



D11

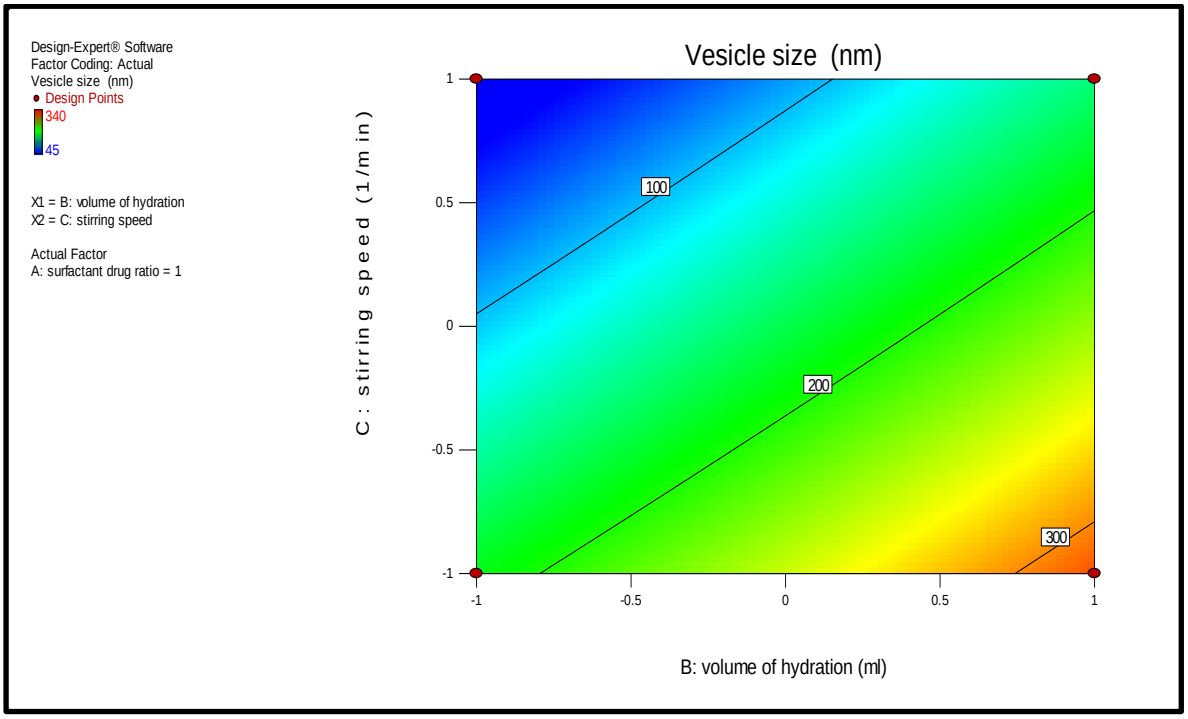
Figure 4.22 Average vesicle size of design batches

Statistical evaluation of factorial batches:-

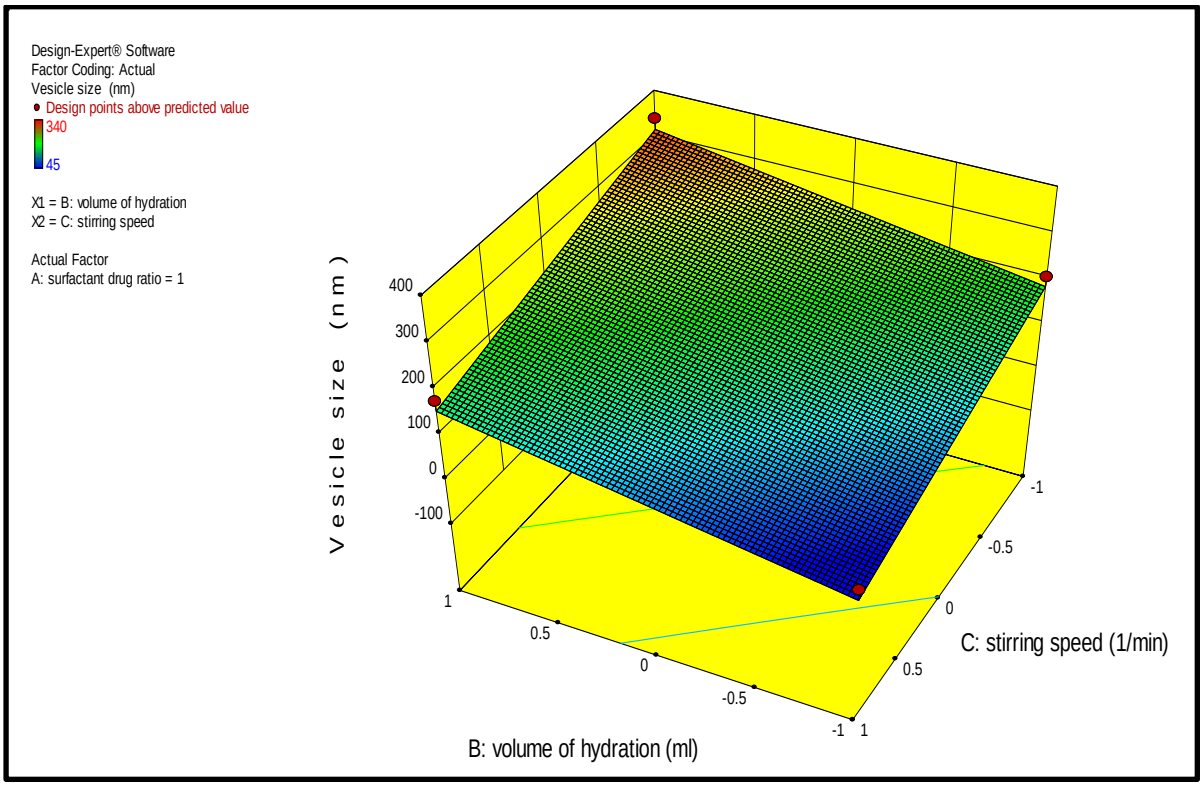
Table 4.38Summary of regression analysis and ANOVA of response R1 (Average vesicle size in nm)

R- Squared	0.8443
Adjusted R-squared	0.6107
Std. Dev	60.45
Mean	170.45

Source	**Sum of squares**	**df**	**Mean square**	**F Value**	**p-value Prob> F**	
Model	79251.50	6	13208.58	1165.46	< 0.0001	Significant
A-span-60 drug ratio	0.50	1	0.50	0.044	0.8471	
B-volume of hydration	22684.50	1	22684.50	2001.57	< 0.0001	
C-stirring speed	55112.00	1	55112.00	4862.82	< 0.0001	
AB	1404.50	1	1404.50	123.93	0.0016	
AC	32.00	1	32.00	2.82	0.1915	
BC	18.00	1	18.00	1.59	0.2967	
Curvature	14581.23	1	14581.23	1286.58	< 0.0001	
Residual	34.00	3	11.33			
Pure Error	8.00	1	8.00	0.62	0.5149	Not significant
Cor Total	26.00	2	13.00			



(a)



(b)

Figure 4.23 Response surface graphs (a) Contour plot (b) 3-D plot for average vesicle size

Final Equation in Terms of Coded Factors for average vesicle size:

R1 = +170.45+0.25*A+53.25*B −83*C+13.25*AB+2*AC+1.5BC……eq. (1)

Interpretation

The linear equation (1) generated after the statistical analysis exemplifies the quantitative effect of factors or independent variables (A), (B) & (C) on the response R1 i.e. average vesicle size. In the equation, the coefficient of the factor reflects the importance of that particular factor where coefficient associated with more than one factor reflects the interaction among the factors. The correlation coefficient R square value was found to be 0.8443 indicated good fit of the linear equation. All of the responses showed a non-significant lack of fit ($p > 0.1$), showing the adequacy of the model fit when fitted in the linear equation model. From the results of ANOVA, p value was found to be less than 0.05 indicated that all the three factors have a significant effect on the response (average vesicle size) (table 4.34). The response surface plots (contour plot and 3 –D response surface plot) as shown in figure 4.22 (a) and 4.22 (b) respectively were drawn using design expert software version 9.0 on the basis of equation generated to estimate and understand the effect of the independent variables on the average vesicle size in a better way.

From the linear equation (1) and response surface plots, it can be concluded that the Factors A and B showed positive effect while Factor C had negative effect on average vesicle size. The major negative effect of Factor C (Stirring speed) on average vesicle size could be due to shear force which increases with increase in stirring speed and hence reduces the vesicle size. The little positive effect of Factor A (Drug: Span-60 ratio) which could be due to higher concentration of span-60 which remains in the solution and forms aggregates and hence increases the vesicle size. The major positive effect of Factor B (Volume of hydration) could be due to higher amount of buffer available for vesicle arrangement, where the aqueous media entrap inside the vesicle as a result of this vesicle size increases. Value of coefficient of interaction between the factor B and C has shown its remarkable effect on average vesicle size.

4.10.4: Response R2 (% Drug entrapment efficiency EE)

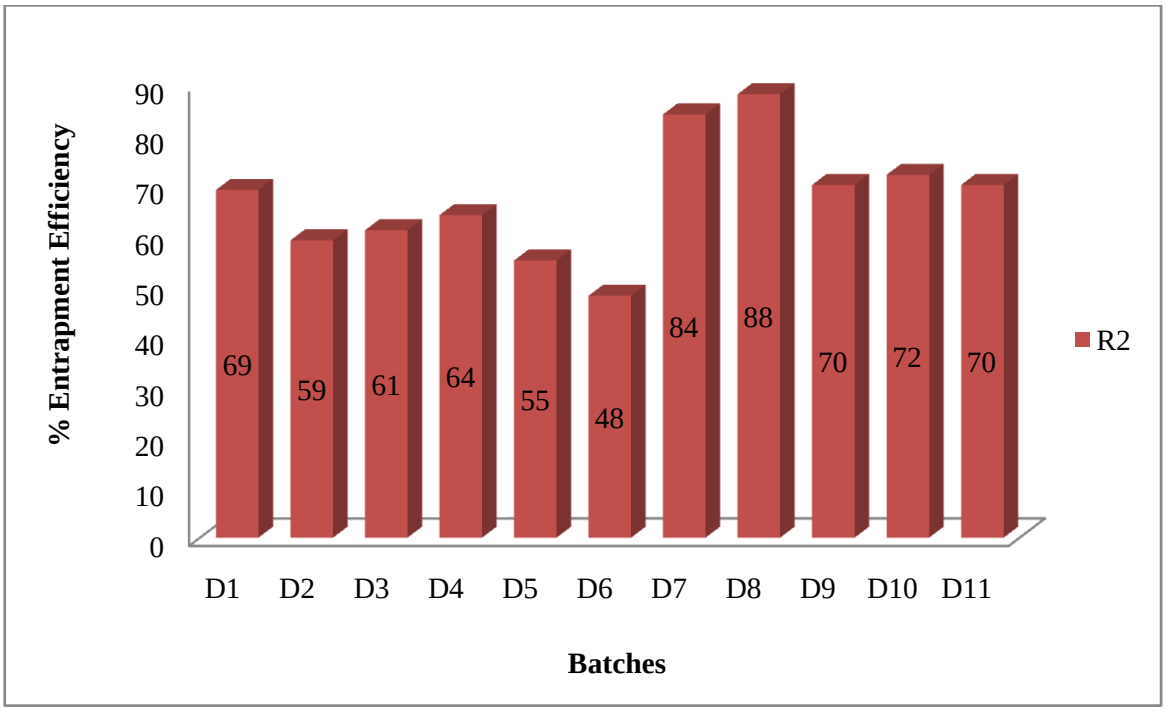


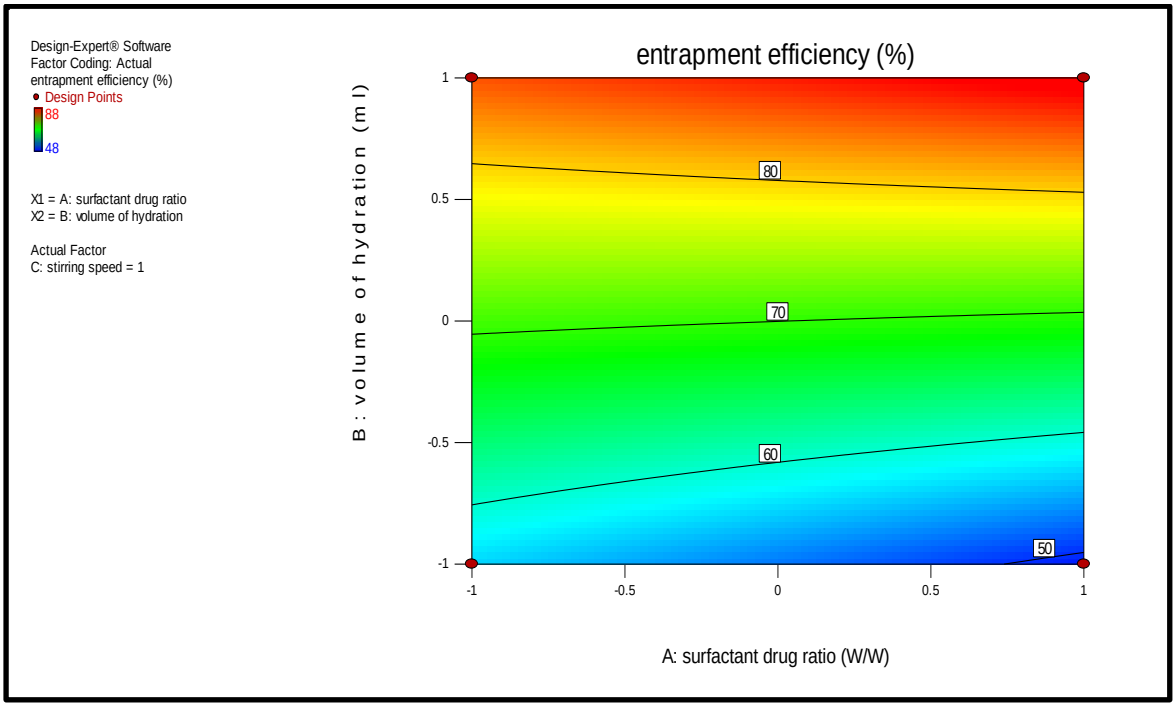
Figure 4.24 Comparative chart of % drug entrapment efficiency of factorial batches

Statistical evaluation of factorial batches:-

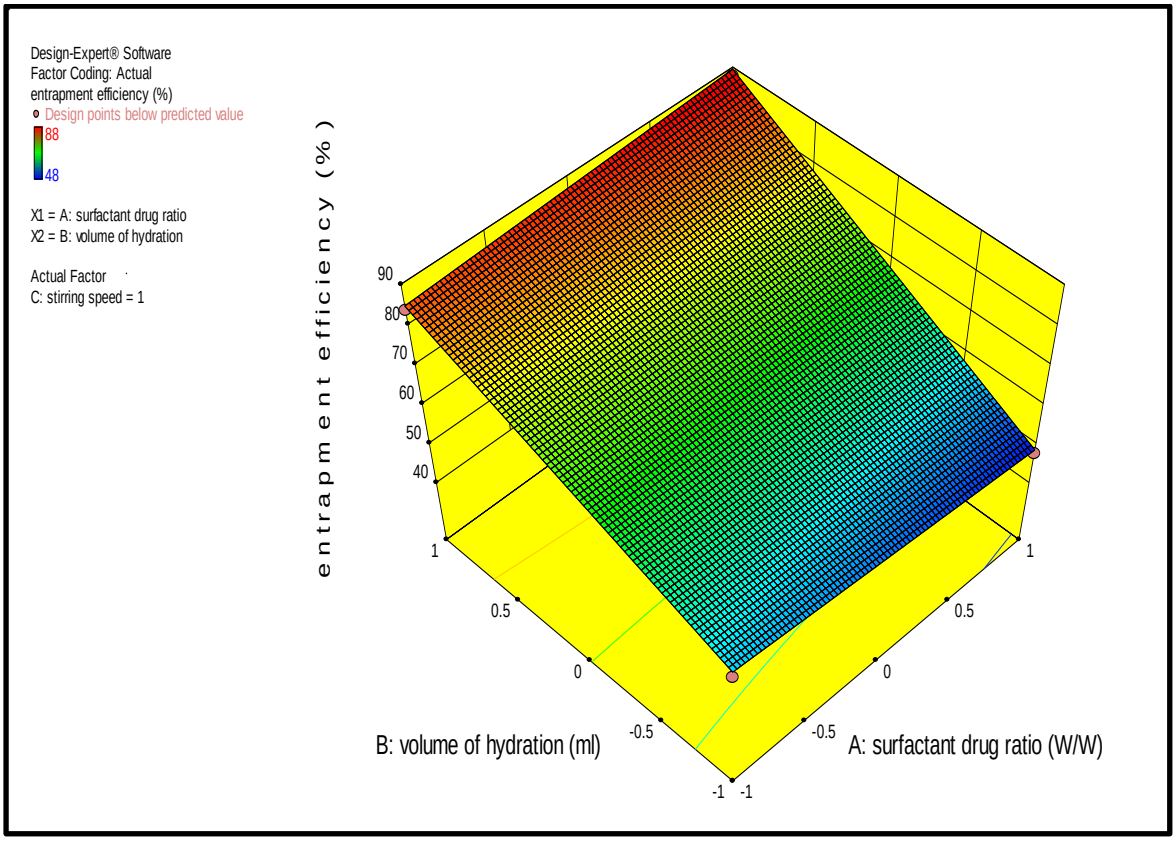
Table 4.39 Summary of Regression analysis and ANOVA response R2 (entrapment efficiency in %)

R- Squared	0.9635
Adjusted R-squared	0.9089
Std. Dev	3.56
Mean	67.27

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	1339.50	6	223.25	211.50	0.0005	Significant
A-span-60 drug ratio	12.50	1	12.50	11.84	0.0412	
B-volume of hydration	544.50	1	544.50	515.84	0.0002	
C-stirring speed	60.50	1	60.50	57.32	0.0048	
AB	72.00	1	72.00	68.21	0.0037	
AC	2.00	1	2.00	1.89	0.2624	
BC	648.00	1	648.00	613.89	0.0001	
Curvature	47.52	1	47.52	45.01	0.0068	
Residual	3.17	3	1.06			
Pure Error	0.50	1	0.50	0.38	0.6026	Not significant
Cor Total	2.67	2	1.33			



(a)



(b)

Figure 4.25: Response surface graphs (a) Contour plot (b) 3-D plot for % drug entrapment efficiency

Final Equation in Terms of Coded Factors for % entrapment efficiency:

R2 = +67.27-1.25*A+8.25*B+2.75*C+3*AB+0.5*AC+9BC……eq. (2)

Interpretation

The linear equation (2) generated after the statistical analysis exemplifies the quantitative effect of factors or independent variables (A), (B) & (C) on the response R2 i.e. % drug entrapment efficiency. The correlation coefficient R square value was found to be 0.9653 indicated good fit of the linear equation. All of the responses showed a non-significant lack of fit (p> 0.1), showing the adequacy of the model fit when fitted in the linear equation model From the results of ANOVA, p value was found to be less than 0.05 indicated that all the three factors have a significant effect on the response (% EE). Just like effect on average vesicle size, factor B and C showed positive effect while factor A showed negative effect on entrapment efficiency. Higher value of coefficient of factor B has shown that it has the maximum effect on the % drug entrapment efficiency among all other factors. The response surface plots (contour plot and 3 –D response surface plot) as shown in figure 4.24 (a) and 4.24 (b) respectively were drawn and took two more important factors on X and Y axis while third least affecting factor was kept at constant higher level (i.e. 2500 rpm stirring speed). The plot clearly shows that drug: span-60ratio has most significant effect on % drug entrapment efficiency as compared to volume of hydration.

Drug entrapment efficiency can also be correlated with vesicle size. Due to the increase in vesicle size by factors B and C entrapment efficiency also increases. The reason for increase in % drug EE with increase in the concentration of span-60 is that it would provide more amount of non polar chain which can occupy more amount of drug in between the bi-layer of vesicles. Factor C (stirring speed) was kept constant at high level in order to achieve the % drug entrapment efficiency with highest desirability. Values of coefficient of interaction between the factors also have some effect on the response but were not found to be significant.

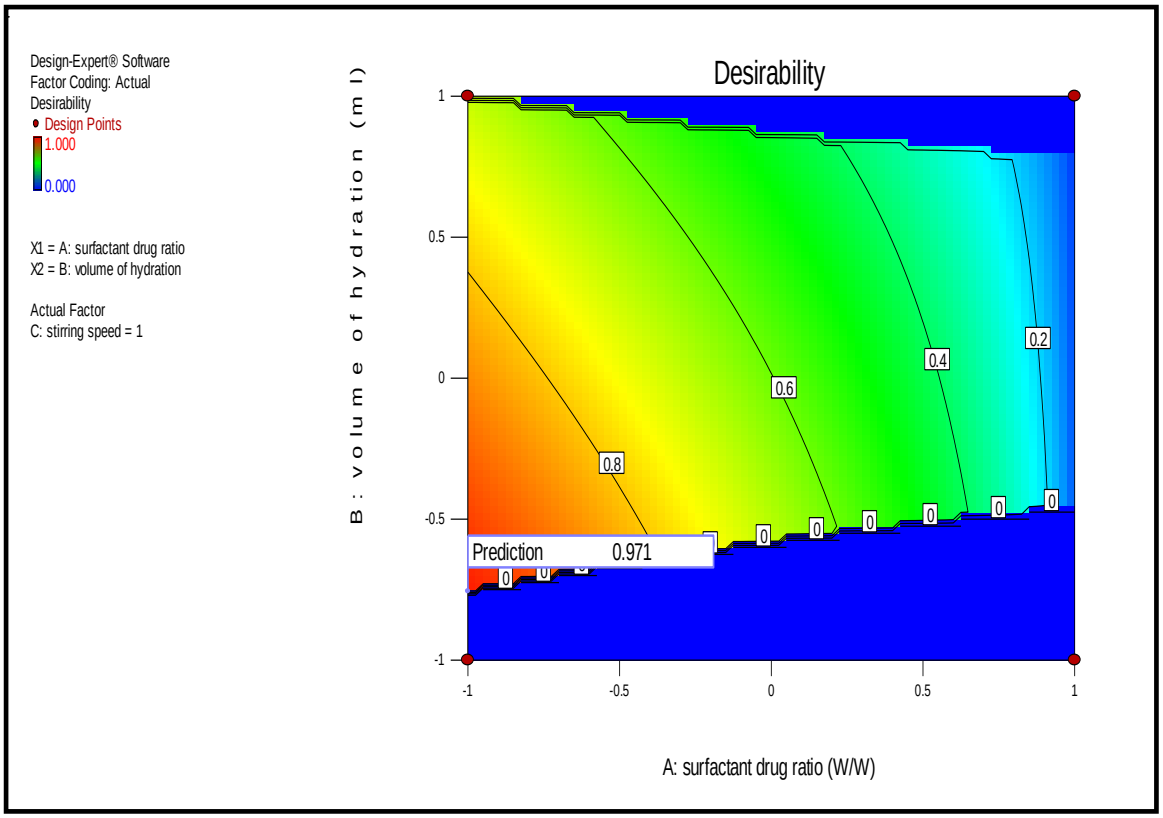


Figure 4.26 Desirability graph

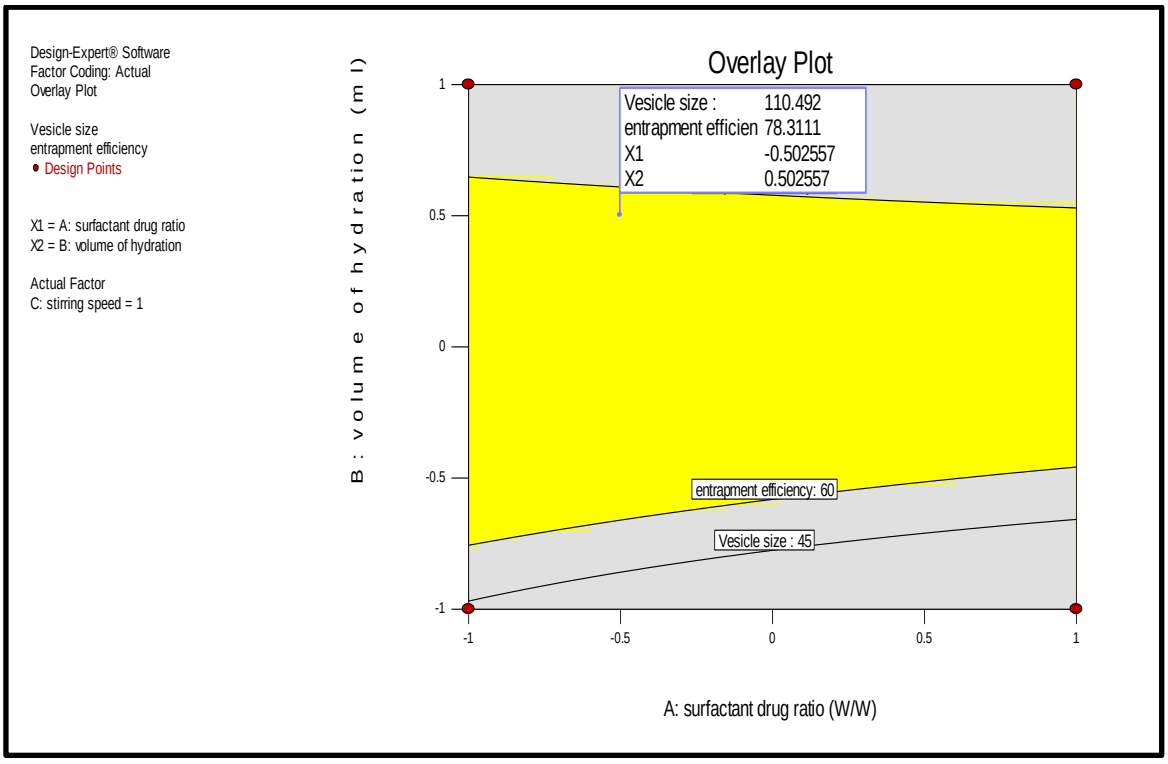


Figure 4.27 Overlay plot

Table 4.40 List of solutions for achieving highest desirability done by design expert software

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:surfactant drug ratio	minimize	-1	1	1	1	3
B:volume of hydration	is in range	-1	1	1	1	3
C:stirring speed	is in range	-1	1	1	1	3
Vesicle size	minimize	45	200	1	1	3
entrapment efficiency	is in range	60	85	1	1	3

Solutions

Number	surfactant drug ratio	volume of hydration	stirring speed	Vesicle size	entrapment efficiency	Desirability	
1	-1.000	-0.756	1.000	53.832	60.000	0.971	Selected
2	-1.000	-0.748	1.000	54.161	60.113	0.970	
3	-1.000	-0.758	0.992	54.393	60.000	0.969	
4	-0.988	-0.753	1.000	53.849	60.002	0.968	
5	-0.991	-0.744	1.000	54.254	60.142	0.967	
6	-1.000	-0.726	1.000	55.090	60.432	0.967	
7	-1.000	-0.763	0.980	55.309	60.000	0.966	
8	-0.971	-0.750	1.000	53.862	60.000	0.964	
9	-1.000	-0.699	1.000	56.189	60.810	0.963	
10	-1.000	-0.768	0.964	56.425	60.000	0.962	

10 Solutions found

Interpretation

From the desirability graph (Figure 4.25), desirability obtained 0.971 indicates that the value of responses obtained in Batch with coded values (-1, -0.756, +1) with the set criteria for all the factors and responses that it was found to be desirable with the predicted value which was shown by the Design expert software 9.0 as shown in table 4.36. Yellow region in the overlay plot (figure 4.26) shows the acceptable region where in the formulation of batches will fulfil the criteria set for optimization.

4.10.5 Formulation of check point batch

In order to validate the equation derived after applying DOE (design of experiments), a check point batch with the predicted levels was prepared and evaluated. (Table 4.41 & 4.42)

Table 4.41 Composition of check point batch

Batch code	Composition		
B12	A (Drug: Span-60 ratio)	B (Volume of hydration)	C (Stirring speed)
Coded (Actual values)	-0.5 (1:5)	-0.5 (32.5)	-0.5 (1375)

Table 4.42 Results of check point batch

Predicted values		Observed values	
Average vesicle size (nm)	**% EE**	**Average vesicle size (nm)**	**% EE**
184	66.20	190	65.58

Results & Discussion: The results observed with the check point batch were found to be matched with the predicted values derived from the linear equation. Hence, the design & statistical model which we have incorporated is mathematically valid and one can reach to an optimized point in shortest time with minimum efforts by adopting organized formulation approach.

4.10.6 Selection of best batch:

Best batch was selected on the basis of OZ loaded niosomes with lower vesicle size and maximum % entrapment efficiency.

Batch D13 with coded values (-0.5, +0.5, +1), actual values (1:5, 75, 2500) obtained from overlay plot was found to have 110.49 nm average vesicle size, 78.31% drug entrapment efficiency along with good desirability. Hence, batch D13 was selected as an optimized batch.

4.10.7 Formulation of scale up batch

A scale up batch D14 was prepared by taking the composition of optimized batch D13 as shown in table 4.43. As per the regulatory guideline, 10 times scale up was done.

Table 4.43 Composition of scale up batch D14

Batch	Drug conc. (mg)	Span 60 Conc. (mg)	Drug: Span 60 ratio (w/w)	DCP conc. (mg)	PBS 7.4 (ml)	Chloroform (ml)
D14	605.8	2180	1:5	80	750	50

Evaluation

Table 4.44 Results of scale up batch D14

Evaluation parameters	D13	D14 (scale up)
Average vesicle size (nm)	110.49	120.4
% Entrapment efficiency	78.31	77.6

Results & Discussion: From the results of the scale up batch it can be concluded that the average vesicle size, %drug and entrapment efficiency was found to be nearly similar with the lab scale i.e. Batch D13. Hence we can conclude that the optimized formulation is scalable.

Table 4.45 Final risk analysis

Process and formulation variables									
CQAs	**Temp**	**Flow rate**	**Span-60: Cholesterol ratio**	**Charge Inducer (DCP)conc.**	**Needle size**	**Sonication**	**Volume of hydration**	**Drug: span-60 ratio**	**Stirring speed**
Vesicle Size	*Low	*Low	*Low	*Low	*Low	*Low	*Low	*Low	*Low
% EE	Low	Low	*Low	Low	Low	*Low	*Low	*Low	*Low
Zeta potential	low	Low	Low	*Low	Low	Low	Low	Low	Low

- **Control strategy:** The factors with *Low category have been converted to low risk from high or medium risk. Individual risk management and control strategy is shown in table 4.46.

Table 4.46 Control strategy

	Attribute	Range studied	Optimized range	Purpose of control
Drug: span 60	Ratio	1:4 – 1:8(molar ratio)	1:4 – 1:8(molar ratio)	To ensure % drug entrapment efficiency in range of 65 % - 80 %
PBS 7.4	Volume	10 ml – 100 ml	25 ml -75 ml	To ensure vesicle size < 200 nm and % drug entrapment efficiency between 65 % - 85 %
Stirrer	Stirring speed	1000 rpm – 2500 rpm	1750 rpm – 2500 rpm	To ensure vesicle size < 200 nm

4.11 Preparation and evaluation of niosomal gel:

4.11.1 Optimization of carbopol gel: Carbopol gel of niosomal formulation by using four different concentration were prepared to study its rheological properties and to evaluate effect of gel concentration on diffusion of niosomes and retention of niosomes in rat skin. The drug concentration and other parameters were kept constant. Formulations of all batches and their evaluation were shown in tables 4.46, 4.47 and 4.48. Figure 4.27 shows % drug diffusion of all batches at different time points.

Table 4.47 Composition of niosomal batches with different concentration of carbopol

Ingredients	G1	G2	G3	G4
Drug (mg)	100	100	100	100
Span 60 (mg)	500	500	500	500
DCP (mg)	8	8	8	8
Volume of PBS 7.4+ glycerol solution (ml)	25	25	25	25
Carbopol 940 (%)	0.5	1	1.5	2

Evaluation:

Table 4.48 Effect of batches G1- G4 for % drug diffusion and rheological properties

Batch no	Viscosity (cP)	pH	Spread-ability	Drug content (T) (%)	% drug diffused after 60 minutes
G1	7543	7.2	+	99.20	19.23
G2	8538	7.2	+++	99.40	16.15
G3	9942	7.1	+++	99.30	11.92
G4	10512	7.4	++	99.22	10.76

*Spreadability: + Poor, ++ Good, +++ Very good

*All the batches were having 0.4 % drug concentration and their physical appearance is translucent and cloudy

Table 4.49: Evaluation of batches G1 – G4 for % drug retention

Batch no.	% drug diffused after 120 min (X)	% drug in donor compartment after 120 min (Y)	% drug retained after 120 min Z = T – (X+Y)
G1	30.00	2.72	67.27
G2	27.30	3.47	69.22
G3	24.23	11.72	64.04
G4	18.84	19.72	61.43

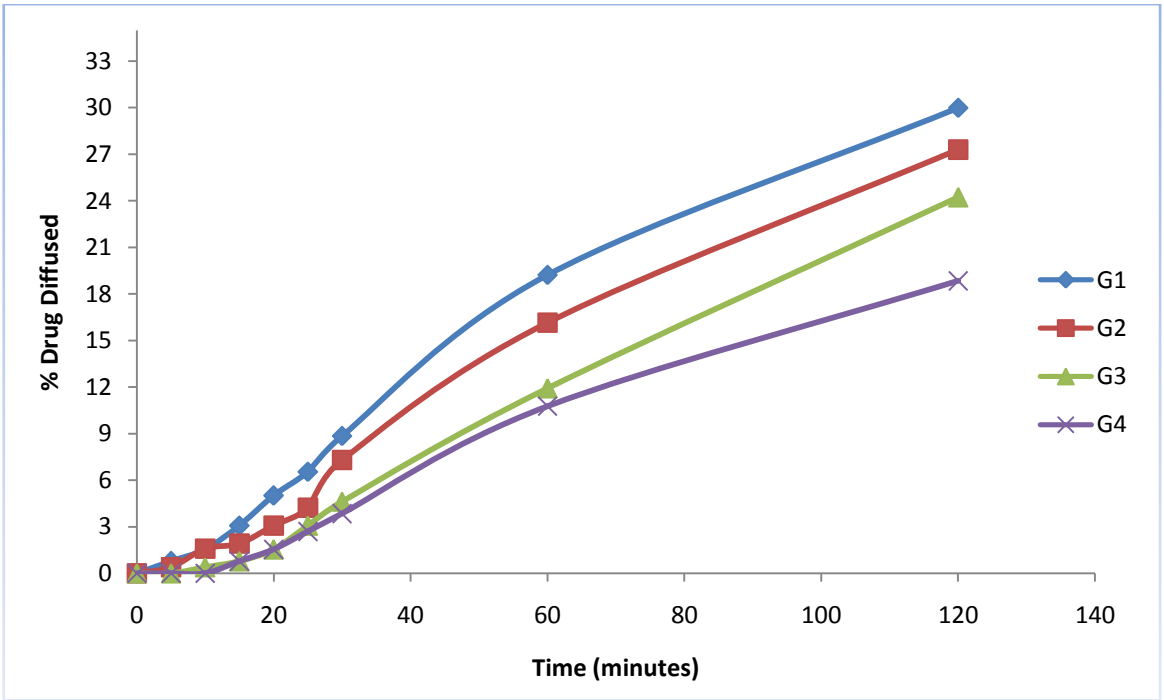


Figure 4.28: Comparison of % drug diffusion of all batches (G1 – G4)

Result and discussion: Niosomal gels showed difference in diffusion profile at various concentration of Carbopol 940. Batch G1 with 0.5 % Carbopol showed highest diffusion after 120 minutes amongst all batches, while batch G4 with 2 % Carbopol showed lowest diffusion. Hence there was inverse relation of drug diffusion with the concentration of gel.

The % drug retention was maximum (69.2 %) in batch G2 with 1 % Carbopol. This showed that optimum concentration of Carbopol gel gives maximum drug retention. The reason for this could be the higher viscosity of batch G3 and G4 which retained niosomal formulation in the gel and could give slower diffusion.

Hence Batch G2 with 1% Carbopol was selected as optimized batch of niosomal gel.

4.11.2 Preparation and evaluation of optimized niosomal gel

The optimized batch obtained from overlay plot is incorporated in 1 % Carbopol gel which is optimized gel concentration. The preparation and evaluation were done by keeping all other parameters as constant. The composition of final batch and it evaluation is as shown in tables 4.49, 4.50 & 4.51.

Table 4.50 Composition of optimized niosomal gel

Batch no	Drug (mg)	Span 60 (mg)	DCP (mg)	PBS 7.4 + glycerol solution (ml)	Carbopol (%)
G5	60	218	8	75	1

Table 4.51 Rheological properties of optimized niosomal gel

Batch no	Appearance	Viscosity (cP)	pH	Spreadability
G5	Translucent & cloudy	8489	7.3	+++

Table 4.52 Permeation study of optimized niosomal gel

Batch no	Drug content (%)	% drug diffused after 60 minutes	% drug diffused after 120 minutes	% drug in donor compartment	% drug retained after 120 minutes
G5	99.70	17.30	25	3.20	70.10

Result and Discussion: The optimized and scalable batch obtained from the overlay plot after applying Full factorial Design which was incorporated in 1 % carbopol gel showed desired rheological properties and % drug retention. Along with other gel formulation, it is stored for stability studies.

4.11.3: Preparation and evaluation of OZ gel

The OZ was accurately weighed and dispersed in the mixture of PBS7.4 and propylene glycol solution, which is subjected to form 1 % carbopol gel by slowly adding weighed quantity of Carbopol. The preparation and evaluations were done by keeping all other parameters as constant. The composition of batch and it evaluation is as shown in tables 4.52, 4.53, 4.54. Figure 4.30 shows the comparison of diffusion between batches G5 and G6.

Table 4.53 Composition of OZ gel

Batch no	Drug (mg)	PBS 7.4 + glycerol solution (ml)	Carbopol (%)
G6	60	75	1

Table 4.54 Rheological properties of OZ gel

Batch no	Appearance	Viscosity (cP)	pH	Spreadability
G6	Transparent with suspended particles	8251	7.3	+++

Table 4.55 Permeation study of OZ gel

Batch no.	Drug content (%)	% drug diffused after 60 minutes	% drug diffused after 120 minutes	% drug in donor compartment	% drug retained after 120 minutes
G6	96.50	5.76	7.69	75.21	5.12

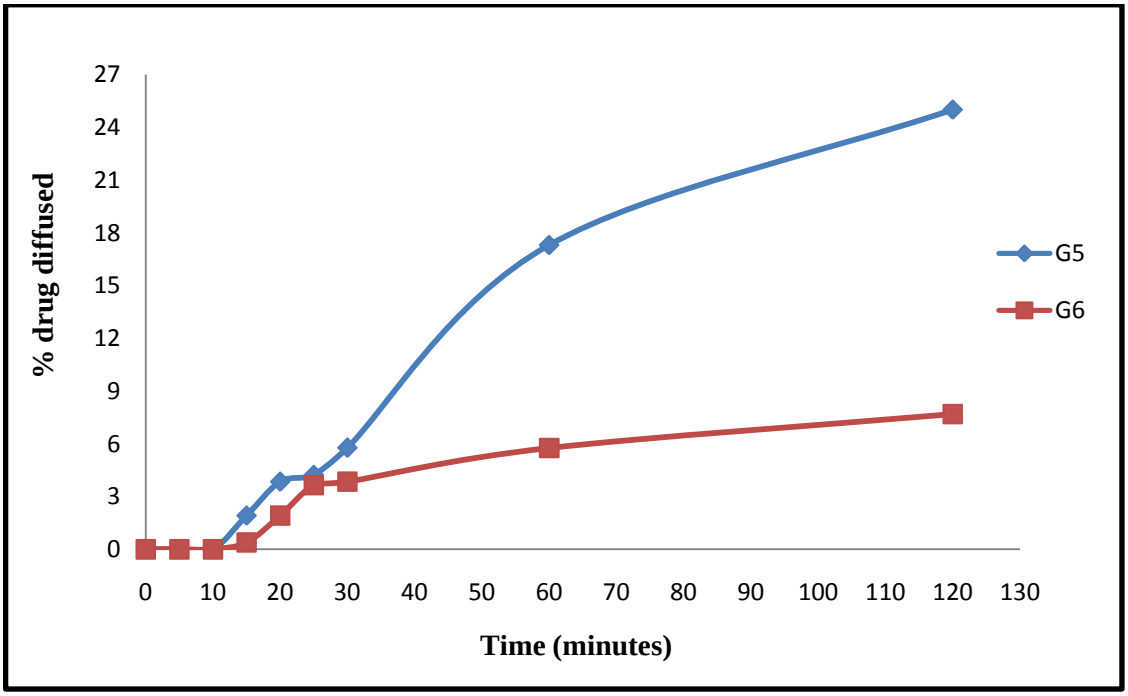


Figure: 4.29 Comparison of diffusion between batches G5 and G6.

Result and Discussion: The Oz gel prepared with the same concentration of drug used in the optimized niosomal gel showed poor drug diffusion and drug retention. The % drug diffusion and retention are 5.76 % and 5.12 % respectively. Also the drug content found was lesser because of poor content uniformity in gel. The reason for such results could be poor solubility of drug.

4.11.2 Stability study of niosomal gel:

All the batches of niosomal gel were stored in wide mouthed glass container at room temperature for the period of one month. The evaluation of all the batches after storage of one moth was shown in tables 4.55 & 4.56.

Table 4.56 Rheological properties and permeation studies after storage of all batches after 1 month

Batch no.	Viscosity (cP)	pH	Spreadability	% drug diffused after 60 minutes	% drug diffused after 120 minutes (X)	% drug in donor compartment (Y)	% drug retained after 120 minutes Z = T – (X+Y)
G1	7444	7.1	+	18	38.5	3.5	65
G2	8321	7.2	+++	15.5	28.2	4.2	67.6
G3	9894	7.1	+++	11	22.23	12.31	65.46
G4	10605	7.3	++	10.2	17.04	21.45	61.51
G5	8410	7.3	+++	17.30	25	3.40	70

*Spreadability:- + Poor, ++ Good, +++ Very good

*All the batches were having 0.4 % drug concentration and their physical appearance is translucent and cloudy

Result and Discussion: After storage of all batches of niosomal gel for period of one month, its rheological properties showed no change. Also there was no significant change in the diffusion profile in all batches. Hence it can be concluded that the niosomal gel do not show leaching of drug are stable at room temperature and there was no leaching of drug from niosomes in its closed container.

4.11.13 Skin irritation test of optimized niosomal gel

Draize's skin irritation test was performed on conscious rabbit skin. The results of 14 days study and its pictures are as shown in table 4.57 and figure 4.30.

Table 4.57 score of skin irritation study

Day	Effect on skin	Score
1	No effect	0
2	No effect	0
3	No effect	0
4	No effect	0
5	No effect	0
6	No effect	0
7	No effect	0
8	No effect	0
9	No effect	0
10	No effect	0
11	No effect	0
12	No effect	0
13	No effect	0
14	No effect	0

*Score: 0 = No redness or edema, 1 = slight redness, 2 = more redness, 3 = redness with edema

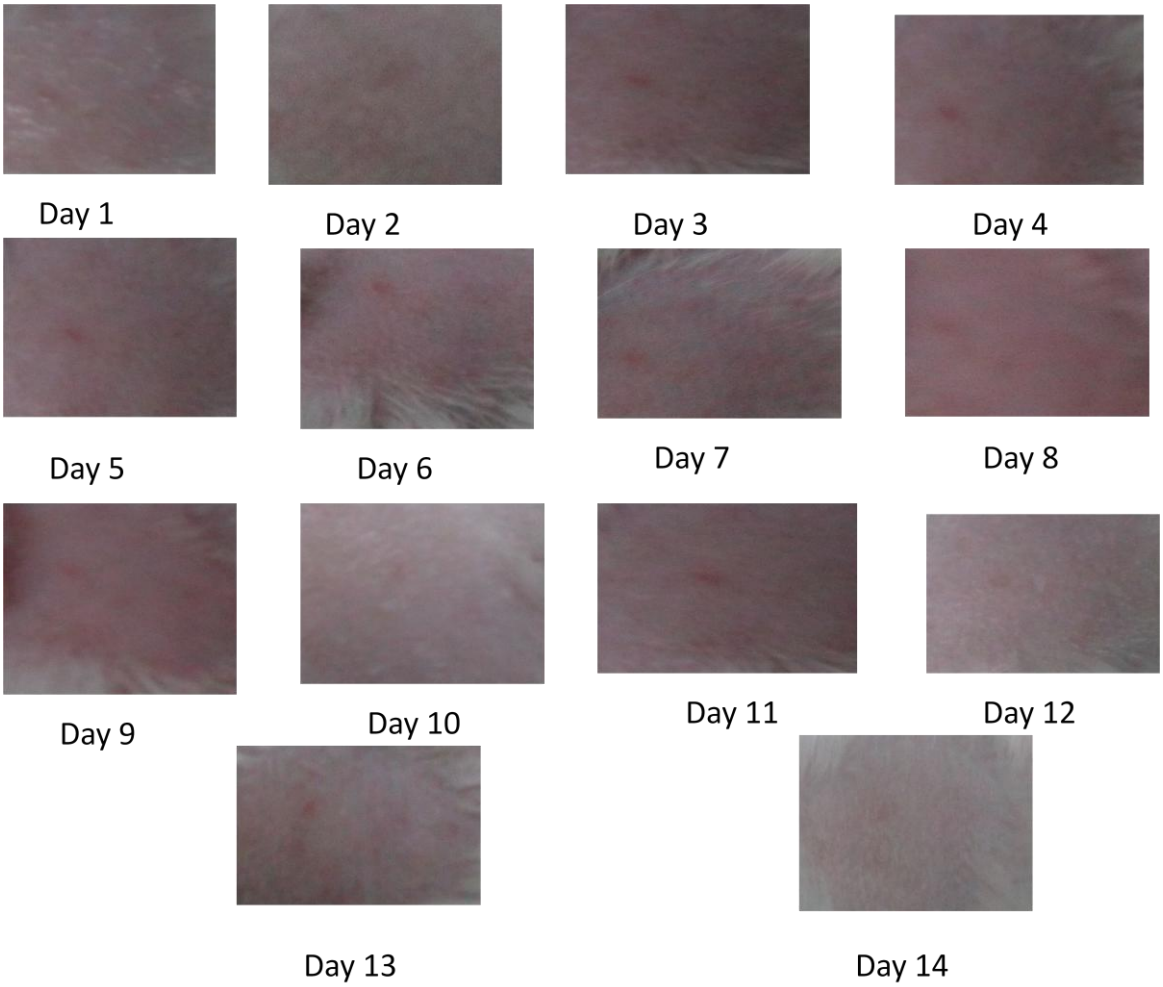


Figure 4.30 skin irritation study

CHAPTER 5

SUMMARY

5. SUMMARY

- In the present research work, OZ niosomes were prepared by two methods e.g. Solvent injection method and thin film hydration method. Preliminary trials were performed for the selection of best method in which solvent injection method was selected over thin film hydration method on the basis of lower aggregation, lesser process time easy recovery and small unilamellar vesicles.
- Then preliminary trials were carried out for screening of important factors on the bases of risk analysis which highlighted the critical factors affecting final formulation. It was found that Drug: span 60 ratio, Volume of hydration and stirring speed were the most critical factors which needs optimization using suitable design. Hence, 2^3 full factorial design was applied for the optimization of OZ niosomes by taking the above three factors as A, B and C respectively and their effect were studied at two levels (-1 & +1). Responses selected were the average vesicle size and % drug entrapment efficiency. From the response surface plot & statistical analysis it was found that the factor B i.e. volume of hydration is having most pronounced effect on both the responses (vesicle size and % entrapment efficiency). Hence, by controlling this factor one can tailor the final formulation characteristics.
- Batch with composition of factors A at -1 level, B at -0.75 level and C at + 1 level fulfilled the targeted criteria's with maximum desirability achieved and the % drug entrapment efficiency obtained was 78.31 % and average vesicle size was 110.49 nm. Hence, this batch was selected as optimized batch. Check point batch showed similarities in the calculated and observed responses indicated that the incorporated mathematical model is valid.
- Scale up is very important from industrial point of view and hence the optimized composition was prepared by taking of 10 times scale up and it was found that the optimized formulation is scalable. Then the final risk analysis was made and control strategy was developed, which could be beneficial for scale up batches.
- The optimized batch was then incorporated in gel and evaluated for various rheological parameters like pH, viscosity and spreadability. The permeability study of niosomal gel was performed by using different concentration of carbopol 940. The

optimized niosomal formulation in 1 % carbopol showed 70.10 % drug retention after 120 minutes of skin permeation study performed on rat skin.

- The gel formulation was tested for skin irritation study as per Draize's skin irritation study on conscious rabbit for period of 14 days, which showed no sign of redness or edema.
- Then the optimized batch of niosomal gel was compared with conventional OZ gel, which showed that the drug retention was only 5.12 % after 120 minutes, which was very low than the optimized niosomal gel.
- Stability study for one month was carried out and it indicated that the stored niosomal solution was not stable at room temperature while it was stable at refrigerated condition. The niosomal gel was found to be stable at room temperature and did not showed significant difference in % drug retention after 1 month. However stability study should be continued further as per ICH requirement.

CHAPTER 6

REFERENCES

6. REFERENCES

(1) Sharma, A.; Saini, S.; Rana, a C. Transdermal Drug Delivery System : A Review. Int. J. Res. Pharm. Biomed. Sci.2013, 4 (1), 286–292.

(2) Brown, M. B.; Traynor, M. J.; Martin, G. P.; Akomeah, F. K. The skin barrier.

(3) Latheeshjlal, L.; Phanitejaswini, P.; Soujanya, Y.; Swapna, U.; Sarika, V.; Moulika, G. Transdermal drug delivery systems: An overview. Int. J. PharmTech Res.2011, 3 (4), 2140–2148.

(4) Dhiman, S.; Singh, T. G.; Rehni, A. K. Transdermal patches: a recent approach to new drug delivery system. Int. J. Pharm. Pharm. Sci.2011, 3 (5), 26–34.

(5) Darwhekar, G.; Jain, D. K.; Patidar, V. K. Formulation and Evaluation of Transdermal Drug Delivery System of Clopidogrel Bisulfate. 2011, 1 (3), 269–278.

(6) Z, P.; C, H. Review on Transdermal Drug Delivery Systems. J. Pharm. Drug Dev.2014, 1(6), 1–10.

(7) Agrawal, V.; Gupta, V.; Ramteke, S.; Trivedi, P. Preparation and evaluation of tubularmicelles of pluronic lecithin organogel for transdermal delivery of sumatriptan. AAPS PharmSciTech2010, 11 (4), 1718–1725.

(8) Kulve, H. Te. Anticipating Market Introduction of Nanotechnology-Enabled Drug Delivery Systems. 2010.

(9) Ramteke, K. H.; Jadhav, V. B.; Dhole, S. N. Microspheres : as carrieres used for novel drug delivery system. IOSR J. Pharm.2012, 2 (4), 44–48.

(10) Peddi, M. G. Molecular Pharmaceutics & Organic Process Research Novel Drug Delivery System : Liquid Solid Compacts. 2013, 1 (3), 1–5.

(11) Schreier, H. Liposomes and niosomes as topical drug carriers: Dermal and transdermal drug delivery. J. Control. Release 1994, 30 (1), 1–15.

(12) Carriers Vesicle based approaches for penetration enhancement in transdermal drug delivery system.

(13) Uchegbu, I. F.; Vyas, S. P. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int. J. Pharm.1998, 172 (1-2), 33–70.

(14) Jain, S.; Jain, V.; Mahajan, S. C. Lipid Based Vesicular Drug Delivery Systems; 2014; Vol. 2014.

(15) Marianecci, C.; Di Marzio, L.; Rinaldi, F.; Celia, C.; Paolino, D.; Alhaique, F.; Esposito, S.; Carafa, M. Niosomes from 80s to present: The state of the art. Adv. Colloid Interface Sci.2014, 205, 187–206.

(16) Kishor Dhamande, R. Z. M. Niosomal drug delivery system - A review. Int. J. Appl. Pharm.2011, 3 (3), 8–11.

(17) Agarwal, A.; Juyal, N.; Kukreti, G.; Yadav, S. Review Article NIOSOMES AS TARGETED DRUG DELIVERY SYSTEMS. 2012, 12 (1).

(18) Sahin, N. O. CHAPTER 4 NIOSOMES AS NANOCARRIER SYSTEMS. 2007, 67–81.

(19) Nvs, M.; Saini, a. Niosomes : a Novel Drug Delivery System. 2011, 1 (3), 498–511.

(20) Manosroi, A.; Chutoprapat, R.; Sato, Y.; Miyamoto, K.; Hsueh, K.; Abe, M.; Manosroi, W.; Manosroi, J. Antioxidant activities and skin hydration effects of rice bran bioactive compounds entrapped in

niosomes. J. Nanosci. Nanotechnol.2011, 11 (3), 2269–2277.

(21) Mishra, N.; Srivastava, V.; Kaushik, A.; Chauhan, V. Formulation and in-vitro evaluation of Niosomes of Aceclofenac. 2014, 3 (3), 337–341.

(22) Abhinav, K.; Lal, P. J.; Amit, J.; Vishwabhan, S. Review on niosomes as novel drug delivery system. Int. Res. J. Pharm.2011, 2 (May), 61–65.

(23) Sankhyan, A.; Pawar, P. Recent trends in niosome as vesicular drug delivery system. J. Appl. Pharm. Sci.2012, 2 (6), 20–32.

(24) Manosroi, a.; Jantrawut, P.; Manosroi, J. Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with diclofenac diethylammonium. Int. J. Pharm.2008, 360 (1-2), 156–163.

(25) Bieke Dejaegher; Heyden, Y. V., Experimental designs and their recent advances in set up, data interpretation, and analytical applications. Journal of Pharmaceutical and Biomedical Analysis 2011, 56, 141-158.

(26) Cicero, a F.; Gaddi, a. Rice bran oil and gamma-oryzanol in the treatment of hyperlipoproteinaemias and other conditions. Phytother. Res.2001, 15 (4), 277–289.

(27) America, T. C. I.; States, U.; Favorite, V. M. γ Oryzanol. 2015, 2–3.

(28) Jeong-Yong Cho; Hyoung Jae Lee; Gee An Kim; Gwi Deok Kim; You Seok Lee; Soo Cheol Shin; Keun-Hyung Park; Moona, J.-H., Quantitative analyses of individual g- Oryzanol (Steryl Ferulates) in conventional and organic brown rice (Oryza sativa L.) Journal of Cereal Science 2012, 55, 337-343.

(29) Umehara, K.; Shimokawa, Y.; Miyamoto, G., Effect of gamma-oryzanol on cytochrome P450 activities in human liver microsomes. Biological & pharmaceutical bulletin 2004, 27(7), 1151-1154.

(30) M. Glavas-Dodov, E. Fredro-Kumbaradzi, K. Goracinova, S. Calis, M. Simonoska, and A. A. Hincal, "5-Fluorouracil in topical liposome gels for anticancer treatment—formulation and evaluation," Acta Pharmaceutica, vol. 53, no. 4, pp. 241–250,2003.

(31) V. Dubey, D.Mishra, and N. K. Jain, "Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery," European Journal of Pharmaceutics and Biopharmaceutics, vol. 67, no. 2, pp. 398–405, 2007.

(32) R.Agarwal,O. P.Katare, and S. P.Vyas, "Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriati drug dithranol," International Journal of Pharmaceutics, vol. 228, no. 1-2, pp. 43–52, 2001.

(33) R. P. Patel, H. Patel, and A. H. Baria, "Formulation and evaluation of liposomes of ketoconazole," International Journal of Drug Delivery Technology, vol. 1, no. 1, pp. 16–23, 2009.

(34) E. Moghimipour, M. Tafaghodi, A. Balouchi, and S. Handali, "Formulation and in vitro evaluation of topical liposomal gel of Triamcinolone acetonide," Research Journal of Pharmaceutical, Biological and Chemical Sciences, vol. 4, no. 1, pp. 101–107, 2013.

(35) Y. R. Hundekar, J. K. Saboji, S. M. Patil, and B. K. Nanjwade, "Preparation and evaluation of diclofenac sodium cubosomes for percutaneous administration," World Journal of Pharmacy and Pharmaceutical Sciences, vol. 3, no. 1, pp. 523–539, 2014.

(36) Lee, J.-S.; Kim, J. S.; Lee, H. G., Oryzanol-loaded calcium pectinate microparticles reinforced with chitosan: Optimization and release characteristics. Colloids and Surfaces B: Biointerfaces 2009, 70, 213–217.

(37) Seetapan, N.; Bejrapha, P.; Srinuanchai, W.; Ruktanonchai, U. R., Rheological and morphological characterizations on physical stability of

gamma-oryzanol-loaded solid lipid nanoparticles (SLNs). Micron 2010, 41, 51-58.

(38) Ghatak, S. B.; Panchal, S. S., Anti-diabetic activity of oryzanol and its relationship with the antioxidant property. Int J Diab Dev Ctries 2012, 32, 185-192.

(39) Tamagawa, M.; Otaki, Y.; Takahashi, T.; Otaka, T.; Kimima, S.; Mrwa, T., Carcinogenicity study of 7-oryzanol in B6C3F1 mice. Food Chemical Toxicology 1992, 30 (1), 49-56.

(40) Lerma-García, M. J.; Herrero-Martínez, J. M.; Mendonç, E. F. S.-A. C. R. B.; Ramis-Ramos, G., Composition, industrial processing and applications of rice bran goryzanol. Food Chemistry 2009, 115, 389-404.

(41) D.Ambrosio; M., Performances of CN-columns for the analysis of g-oryzanol and its p-coumarate and caffeate derivatives by normal phase HPLC and a validated method of quantitation. Food Chemistry 2013, 138, 2079–2088.

(42) Shawn Madere Nutritional supplement to improve supplementary target protein fraction (tpf) delivery, intracellular absorption and utilization. 2013.

(43) Shirazi, S.; Akrong, J.; Apong, P.; Bashir, R.; Wissent, C. Weight loss formulation. 2012.

(44) Cherukuri, R. S. V.; Cheruvanky, R. Therapeutic uses of an anti-cancer composition derived from rice bran. 2007.

(45) Gorsek, W. F. Cholesterol treatment formulation. 2003.

(46) Rao, K. V. S. A.; Rao, B. V. S. K.; Thengumpillil, N. B. K. Process for the isolation of oryzanols from rice bran oil soap stock. 2002.

(47) Cherukuri, R. S. V.; Cheruvanky, R.; Lynch, I.; McPeak, D. L. Process for obtaining

micronutrient enriched rice bran oil. 1999.

(48) Manosroi, A.; Chutoprapat, R.; Abe, M.; Manosroi, W.; Manosroi, J. Transdermal Absorption Enhancement of Rice Bran Bioactive Compounds Entrapped in Niosomes. AAPS PharmSciTech2012, 13 (1), 323–335.

(49) Balakrishnan, P.; Shanmugam, S.; Lee, W. S.; Lee, W. M.; Kim, J. O.; Oh, D. H.; Kim, D. D.; Kim, J. S.; Yoo, B. K.; Choi, H. G.; et al. Formulation and in vitro assessment of minoxidil niosomes for enhanced skin delivery. Int. J. Pharm.2009, 377 (1-2), 1–8.

(50) Bragagni, M.; Mennini, N.; Ghelardini, C.; Mura, P. Development and characterization of niosomal formulations of doxorubicin aimed at brain targeting. J. Pharm. Pharm. Sci.2012, 15 (1), 184–196.

(51) Budai, L.; Kaszás, N.; Gróf, P.; Lenti, K.; Maghami, K.; Antal, I.; Klebovich, I.; Petrikovics, I.; Budai, M. Liposomes for topical use: A physico-chemical comparison of vesicles prepared from egg or soy lecithin. Sci. Pharm.2013, 81 (4), 1151–1166.

(52) Casiraghi, A.; Grigoli, M.; Cilurzo, F.; Gennari, C. G. M.; Rossoni, G.; Minghetti, P. The Influence of the Polar Head and the Hydrophobic Chain on the Skin Penetration Enhancement Effect of Poly (Ethylene Glycol) Derivatives. AAPS PharmSciTech2012, 13 (1), 247–253.

(53) Dill, K. a; Flory, P. J. Molecular organization in micelles and vesicles. Proc. Natl. Acad. Sci. U. S. A.1981, 78 (2), 676–680.

(54) Guinedi, A. S.; Mortada, N. D.; Mansour, S.; Hathout, R. M. Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. Int. J. Pharm.2005, 306 (1-2), 71–82.

(55) Lingan, M. A.; Sathali, a A. H.; Kumar, M. R. V.; Gokila, a. Formulation

and Evaluation of Topical Drug Delivery System Containing Clobetasol Propionate Niosomes. Sci. revirews Chem. Commun.2011, 1 (1), 7–17.

(56) Manosroi, a.; Jantrawut, P.; Manosroi, J. Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with diclofenac diethylammonium. Int. J. Pharm.2008, 360 (1-2), 156–163.

(57) Marianecci, C.; Di Marzio, L.; Rinaldi, F.; Celia, C.; Paolino, D.; Alhaique, F.; Esposito, S.; Carafa, M. Niosomes from 80s to present: The state of the art. Adv. Colloid Interface Sci.2014, 205, 187–206.

Project report

ORIGINALITY REPORT

13%	7%	7%	17%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Institute of Technology, Nirma University Student Paper	5%
2	www.docstoc.com Internet Source	2%
3	www.ijrpc.com Internet Source	1%
4	www.ijntps.org Internet Source	1%
5	ajaxprakash.blogspot.in Internet Source	<1%
6	imperialpharmajournals.com Internet Source	<1%
7	Nefise Ozlen Sahin. "Niosomes as Nanocarrier Systems", Nanomaterials and Nanosystems for Biomedical Applications, 2007 Publication	<1%
8	Ranade, . "Transdermal drug delivery", Drug Delivery Systems Second Edition, 2003. Publication	<1%