

# “FORMULATION AND EVALUATION OF LIPOSOMAL TOPICAL GEL OF LINEZOLID FOR THE TREATMENT OF SKIN INFECTION”

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## ABSTRACT: <sup>[1]</sup>

Liposome is the most common and well-investigated nano carriers for targeted drug delivery. They have improved therapies for a range of biomedical applications by stabilizing therapeutic compounds. Liposome is defined as phospholipids vesicles consisting of one or more concentric lipid bilayers enclosing discrete aqueous spaces. Topical drug delivery is an attractive route for local and systemic treatment. Linezolid is a synthetic antibiotic, an oxazolidinone derivative that is active against bacteria. Linezolid is available commercially as tablets and injections only in spite of its well known adverse effects including nausea, vomiting, headache, diarrhea and abdominal discomfort. Oral Linezolid infectious diseases caused mainly by aerobic gram positive bacteria, certain negative bacteria and anaerobic cannot be taken in conjugation with a number of medications. They are capable of incorporate a variety of hydrophilic and hydrophobic drugs to enhance the accumulation of drug at the administration site and to reduce side effects. The objective of the present study was to develop a Linezolid loaded liposomal gel for better anti-bacterial activity by sustaining the drug release and reduces adverse effects. Linezolid is a synthetic antibacterial agent of a new class of Oxazolidinone, which has more effective clinical utility in treatment of infectious diseases compared to Vancomycin, Methicillin. Linezolid liposome were prepared by thin film hydration technique using soya lecithin, Stearic acid, cholesterol and drug in different weight ratios. They were evaluated for particle size, entrapment efficiency and *in vitro* drug release.

**KEYWORDS:** liposomal drug delivery, nano carriers, in vivo, in vitro.

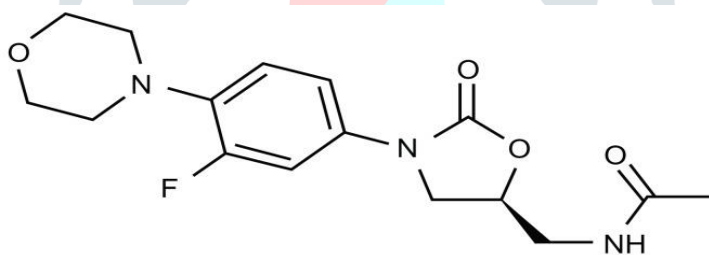
## INTRODUCTION: <sup>[2]</sup>

The objective of the present study was to develop a Linezolid loaded liposomal gel for better anti bacterial activity by sustaining the drug release and reduces adverse effects. Linezolid is a synthetic antibacterial agent of a new class of Oxazolidinone, which has more effective clinical utility in treatment of infectious diseases compared to Vancomycin, Methicillin. Linezolid liposome's were prepared by thin film hydration technique using soya lecithin, cholesterol and drug in different weight ratios. Topical drug delivery is an attractive route for local and systemic treatment. The delivery of drugs onto the skin is recognized as an effective means of therapy for local dermatological diseases. Linezolid is a synthetic antibiotic, an oxazolidinone derivative that is active against infectious diseases caused mainly by aerobic gram positive bacteria, certain negative bacteria and anaerobic bacteria. Linezolid is available commercially as tablets and injections only in spite of its well known adverse

effects including nausea, vomiting, headache, diarrhea and abdominal discomfort. Oral Linezolid cannot be taken in conjugation with a number of medications. In order to bypass these disadvantages, the liposomal gel formulations have been proposed as topical applications. Topically Linezolid is used in the treatment of skin and soft tissue diseases. To sustain the drug release for a prolonged period of time and minimize the side effects it can be formulated in the form of liposome. Liposomes have been widely used as drug carrier in topical treatment of diseases, especially in dermatology. They are capable of incorporate a variety of hydrophilic and hydrophobic drugs to enhance the accumulation of drug at the administration site and to reduce side effects. However major limitation of using liposome's topically is the liquid nature of preparation. That can be overcome by their incorporation in vehicles such as carbopol 934, propylene glycol.

### 1. LINEZOLID:

Linezolid is an antibiotic used for the treatment of infections caused by Gram-positive bacteria. A large meta-analysis of randomized controlled trials found Linezolid to be more effective than glycopeptides antibiotics (such as vancomycin and teicoplanin) and beta-lactam antibiotics in the treatment of skin and soft tissue infections (SSTIs) caused by Gram-positive bacteria, and smaller studies appear to confirm its superiority over in the treatment of all serious Gram-positive infections. In the treatment of diabetic foot infections, Linezolid appears to be cheaper and more effective than vancomycin.



#### A. CHEMICAL STRUCTURE OF LINEZOLID

Linezolid is a synthetic antibacterial agent of a new class of Oxazolidinone, which has more effective clinical utility in treatment of infectious diseases compared to Vancomycin, Methicillin. The in vitro spectrum of activity of Linezolid also includes certain Gram-negative bacteria and anaerobic bacteria. Linezolid is rapidly and extensively absorbed after oral dosing. Maximum plasma concentrations are reached approximately 1 to 2 hours after dosing, and the absolute bioavailability is approximately 100%. It has 31% protein binding. Linezolid is primarily metabolized by oxidation of the morpholine ring which results in two inactive ring-opened carboxylic acid metabolites: the amino ethoxyacetic acid metabolite (A), and the hydroxyethyl glycine metabolite.

#### B. MECHANISM OF ACTION:<sup>[3,4]</sup>

Linezolid is, used the treatment of infections caused by aerobic Gram-positive bacteria. The in vitro spectrum of activity of Linezolid also includes certain Gram-negative bacteria and anaerobic bacteria. Susceptible organisms include Methicillin- and vancomycin-resistant staphylococci, vancomycin-resistant Enterococci, penicillin-

resistant pneumococcal and anaerobes. Oxazolidinone inhibit protein synthesis by binding at the P site at the ribosomal 50S subunit. Resistance to other protein synthesis inhibitors does not affect oxazolidinone activity. However rare development of oxazolidinone resistance cases, associated with 23S rRNA alterations during treatment have been reported. Linezolid inhibits bacterial protein synthesis through a mechanism of action different from that of other antibacterial agents; therefore, cross-resistance between Linezolid and other classes of antibiotics is unlikely.

### **C. PREPARATION OF LINEZOLID LIPOSOME'S: [5]**

The preparation of liposome's with Soybean lecithin was prepared by dried thin film hydration technique using rotary evaporator. Different weight ratios of soya lecithin, cholesterol and Stearic acid were weighed and dissolved in chloroform and methanol mixture (2:1) in 250 ml round bottom flask. A thin film was formed on evaporating organic solvent under vacuum using Rota evaporator at 45-60°C. The dried lipid film was hydrated with 10 ml of phosphate buffer solution (pH 6.8) which containing drug. The dispersion was left undisturbed at room temperature for 2-3 hour to allow complete swelling of the lipid film and hence to obtain vesicular dispersion.

### **D. METHODS FOR PREPARATION OF LIPOSOME: [6]**

The main goal of an ideal method of liposome formulation is to obtain efficient drug entrapment, narrow particles size distribution and long term stability of liposome products. The general procedure for all methods of liposome preparation involves hydrating of the lipid, followed by sizing of the particles and removing of the non encapsulated drug. There are two types used for the preparation of liposome: passive loading mechanical dispersion methods and active loading methods. The most common used methods in the preparation for liposome are: thin-film hydration method, micro emulsification, sonication, and membrane extrusion, freeze thawed method, ether injection method, ethanol injection method, reverse phase evaporation method, dehydration-rehydration, and calcium-induced fusion method 9,44,46,48. In the passive loading method the drug is encapsulated by introducing an aqueous phase of a water-soluble drug or an organic phase of a lipid-soluble drug before or at some stage during the preparation of the liposome. The high drug encapsulation efficiency can be achieved by using passive loading method for lipid-soluble drugs with a high affinity to the lipid membrane. In the active loading method, the drugs can be loaded by creating diffusion gradients for the ions or drugs across the external and internal aqueous phases. The classification of liposome based on methods for their preparation depends on using the organic solvent, obtaining liposome with different lamellarity, transforming the size and applications of the liposome. The phospholipids play important role in the preparation of liposome, as well as in their stability 48. In table 2 are presented most commonly used phospholipids.

## 1. Thin-Film Hydration Method:

The thin-film hydration procedure is the most common and simple method for preparation of MLV by dissolving the phospholipids in the organic solvents: dichloromethane, chloroform, ethanol and chloroform-methanol mixture (2:1 v/v; 9:1 v/v; 3:1 v/v). A thin and homogeneous lipid film is formed when solvent is evaporated under vacuum at the temperature: 45-60 °C. Nitrogen gas is involved in order to completely remove the residual solvent. A solution of distilled water, phosphate buffer, phosphate saline buffer at pH 7.4 and normal saline buffer are used in hydration step. The time for the hydration process varied from 1 h to 2 h at the temperature 60-70 °C. In order to obtain full lipid hydration, the liposomal suspension is left overnight at 4 °C. The thin-film hydration method can be used for all different kinds' of lipid mixtures. The main drawbacks of the method are related to low encapsulation, difficulty of scaling up and the size distribution is heterogeneous.

## 2. Injection Methods:

### 2.1. Ether Injection Method:

In ether injection method a solution of lipids is dissolved in ether or diethyl ether/methanol mixture which is slowly injected to an aqueous solution of the material to be encapsulated. The subsequent removal of the organic solvent under reduced pressure leads to the formation of liposome. The main disadvantage of the method is heterogeneous population and the exposure of compounds to be encapsulated to organic solvents or high temperature.

### 2.2. Ethanol Injection Method:

In ethanol injection method the ethanol lipid solution is rapidly injected to a vast excess of preheated distilled water or TRIS-HCl buffer 5. The incorporation of the drug in liposomal vesicle depends on its hydrophilic/hydrophobic character. Nimesulide as lipid soluble component incorporates better in liposome than 5-fluorouracil which migrates to external aqueous phase. The main advantage of ethanol injection method is including of non harmful solvent as ethanol, as well as easy scale up of the method. The possibility of formation of azeotrope with water reduces its applicability.

### 2.3. Sonication Method:

The sonication method is based on size transformation and involves the subsequent sonication of MLVs prepared by thin-film hydration method, using sonic energy usually under an inert atmosphere including nitrogen or argon. The sonication method enables homogenous dispersion of small vesicles using bath type or probe type sonicator with a potential for greater tissue penetration. The probe tip sonicator delivers high energy to the lipid suspension. The possibility of overheating of the lipid suspension causes degradation. Sonication tips tend to release titanium particles into the lipid suspension which must be removed by centrifugation prior to use. The bath sonicator is the

most widely used instrumentation for preparation of SUV. They are used for large volume of dilute lipids. The oxidation of unsaturated bonds in the fatty acid chains of phospholipids and hydrolysis to lysophospholipids and free fatty acids, as well as denaturation of thermo labile substances and very low encapsulation efficiency of internal volume are the main drawbacks of the method.

#### **2.4 High-Pressure Extrusion Method:**

MLVs prepared by thin-film hydration method are repeatedly passed through filters polycarbonate membranes reducing the liposome size in high-pressure extrusion method. The liposome is prepared using thin-film hydration method subsequently using an extruder for ten cycles to obtain extruded liposome with uniform diameters.

#### **2.5. Reverse-Phase Evaporation Method:**

The reverse-phase evaporation method is used with the organic solvents such as diethyl ether/isopropyl ether or mixture of diethyl ether and chloroform (1:1 v/v)<sup>5</sup> and a mixture of chloroform methanol (2:1 v/v) containing phospholipids. The organic phase should be immiscible with aqueous phase, thus an oil/water emulsion is created. Phosphate buffer saline or citric Na<sub>2</sub>HPO<sub>4</sub> buffer is added to aqueous phase with aim to improve the efficiency of liposome formulations<sup>5</sup>. The formation of liposome is allowed by continued rotary evaporation of the organic solvent under vacuum the main advantage of the method is a very high encapsulation rate. The main drawback of the method is the possibility of remaining the solvent in the formulation and it has difficulties to scale up.

#### **2.6 Calcium-Induced Fusion Method:**

The calcium-induced method is based on adding of calcium to SUV. The formation of multilamellar vesicles is as result of fusion. The addition of ethylene diamine tetra acetic acid (EDTA) to the preparations results in the formation of LUV liposome. The preparation of LUV liposome can be obtained only from acidic phospholipids.

#### **2.7 Dehydration-Rehydration Method:**

The method of dehydration-rehydration is used as method for the preparation of liposome, also. The small unilamellar vesicles which are composed of phosphatidylcholine, 1, 2-dioleoyl-3-(tri methyl ammonium) propane, cholesterol and plasmid DNA are prepared by sonication method. The obtained formulation is frozen and left freeze-dried overnight. The formation of multilamellar dehydration-rehydration vesicles containing DNA in their structure due to the bound of the cationic charges of the inner bilayers is as a result of a controlled rehydration of the dry powders.

#### **2.8. Freeze-Thaw Method:**

The method of freezing and thawing is introduced for increasing the trapped volume of liposomal preparations. The freeze-thaw method is dependent on the ionic strength of the medium and the phospholipids concentration. It

influences to a physical disruption of lamellar structure leading to formation of unilamellar vesicles. The unilamellar vesicles are rapidly frozen followed by slow thawing, while the freeze and thawing cycles are repeated. The preparation of MLV propranolol liposome by freeze-thaw method is described in the literature.

### **2.9 Micro fluidization:**

A method based on micro fluidization i.e. micro emulsification is used for the large scale manufacture of liposome. The preparation of antibiotic liposome by thin-layer hydration method followed by sonication with a bath-type sonicator and micro fluidization in order to achieve partial homogenization. The process of micro fluidization is reproducible and yield liposome with good aqueous phase encapsulation.

### **2.10 Supercritical Fluids (SCF) in the Preparation of Liposome:**

Supercritical fluids are introduced in the preparation of liposome to overcome existing problems with conventional methods such as requiring a high amount of toxic organic solvents and limited laboratory scale production. The most common used supercritical fluid in the preparation of liposome in pharmaceutical field is supercritical carbon dioxide. It has several advantages: non-toxicity, non-flammability, recyclable and easy removal from the solvent, operation at moderate temperatures and avoiding degradation of the product in an inert atmosphere. The use of SCF allows controlling of extraction condition by variation of temperature, pressure or adding modifier solvents as co solvents: acetone, ethanol, methanol, dichloromethane and ethyl acetate. A comparison between thin-film hydration method and SCF method. A mixture of phosphatidylcholine, cholesterol and cyclosporin A is dissolved in ethanol followed by pumping supercritical carbon dioxide to the reaction vesicle in SCF method. Distilled water in hydration step in thin-film hydration method is used.

### **2. GELS:-<sup>[7, 8, 9, 10]</sup>**

These are transparent to opaque semisolids containing a high ratio of solvent to gelling agent merge or entangle to form a three-dimensional colloidal network structure. This network limits fluid flow by entrapment and immobilization of the solvent molecules. The network structure is also responsible for a gel resistance to deformation and therefore, its viscoelastic properties. Gels tend to be smooth, elegant, non greasy and produce cooling effect and utilize better drug release as compared to other semi-solid formulation.

#### **A. Introduction to gels:**

Gels are becoming more popular due to ease of application and better percutaneous absorption. The term gel was introduced in the late 1800 to name some semisolid material according to pharmacological, rather than molecular criteria. The USP defines gels as semisolid systems consisting of either suspensions made up of small inorganic particles, or large organic molecules interpenetrated by a liquid, where the gel mass consists of a network of small discrete particles, the gel is classified as a two-phase system. Gels are usually clear transparent semisolids containing the solublizer active substance. Single-phase gels can be described as three-dimensional networks

formed by adding macromolecules such as proteins, polysaccharides, and synthetic macro molecules to appropriate liquids. In pharmaceutical applications, water and hydro alcoholic solutions are most common. Many polymer gels exhibit reversibility between the gel state and sol, which is the fluid phase containing the dispersed or dissolved macromolecule. However, formation of some polymer gels is irreversible because their chains are covalently bonded. The term gel represents a physical state with properties intermediate between those of solid and liquids. However, it is often wrongly used to describe any fluid system that exhibits some degree of rigidity. It is therefore recommended that the term should be restricted to those systems that satisfy the following criteria:-

1. These should be coherent colloidal system of at least two components (the gelling agent and a Fluid component).
2. They should exhibit mechanical properties of the solid state.
3. Each component should be continuous throughout the system.

The term gel is broad, encompassing semisolid of a wide range of characteristics from fairly rigid gelatin slabs, to suspensions of colloidal clays, to certain greases. A gel can be looked upon as being composed of two interpenetrating phase, the gelling agent and a fluid component. Gels are semisolid dosage forms, that are either suspensions of small inorganic particles or large organic molecules interpenetrated with liquid vehicle. In the first case, the inorganic particles form a three-dimensional structure throughout the gel. This forms a biphasic system, as the inorganic particles are not soluble but dispersed uniformly throughout the continuous phase. Large organic molecules tend to exist in solution form as randomly coiled flexible chains. These molecules which consist of natural or synthetic polymers tend to entangle with each other because of their random motion. It is interaction between the units of the colloidal phase, inorganic or organic, that sets up the structural viscosity immobilizing the liquid continuous phase. Thus gels exhibit characteristics intermediate to those of liquid and solids.

## **B. Classification of Gels:-**

Gels are classified mainly by two methods based on:

### **a) Nature of colloid phase**

- i) Inorganic gels
- ii) Organic gels

### **b) Based on nature of solvent**

- i) Aqueous gels
- ii) Non aqueous gels

### c) Based on their microstructure

- i) Covalently bonded polymer networks with completely disordered structures.
- ii) Physically bonded polymer networks, predominantly disordered but containing ordered loci.
- iii) Well-ordered lamellar structures, including gel mesophases formed by inorganic clays.

### C. Gel forming substances:-

Gels forming hydrophilic polymers are typically used to prepare lipid-free semisolid dosage forms, including dental, dermatological, nasal, ophthalmic, rectal, and vaginal gels and jellies. Polymers produce a structural network, which is necessary for the preparation of gels.

### D. Benefits of transdermal gel over other semi solid dosage forms:-

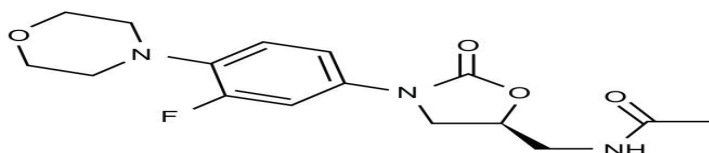
1. Gels have high water content so they hydrate the skin and reduce the skin irritation.
2. Hydrophilic gels are easily removed by gentle rinsing or natural flushing with body fluids.
3. They show thixotropic behavior and have good spread ability.
4. Gels are none staining and compatible with number of excipient.

### E. Limitations of transdermal gel:-

1. Skin irritation of contact dermatitis may occur due to the drug.
2. Poor permeability of some drugs through the skin.
3. Possibility of allergenic reactions.
4. Drugs of larger particle size not easy to absorb through the skin.
5. The drug release is unpredictable and there is probability of loss of drug.

## 3. DRUG AND EXCIPIENT PROFILE <sup>[11]</sup>

### 3.1. Linezolid:



Chemical structure of Linezolid



<b>Molecular Formula</b>	C <sub>16</sub> H <sub>20</sub> FN <sub>3</sub> O <sub>4</sub>
<b>Molecular Weight</b>	337.4
<b>IUPAC Name</b>	N-[[[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl]acetamide
<b>Description</b>	White to off white crystalline powder
<b>Melting Point</b>	181°C
<b>Solubility</b>	Soluble in chloroform and slightly soluble in methanol
<b>Category</b>	Antibacterial
<b>Indication</b>	For the treatment of bacterial infections caused by susceptible strains of vancomycin resistant <i>Enterococci faecium</i> , <i>Staphylococcal aureus</i> (Methicillin resistant and susceptible strains), <i>Streptococcus pneumoniae</i> , <i>Streptococcus pyogenes</i> , <i>Streptococcusagalactiae</i> .
<b>Pharmacokinetic</b>	Bioavailability : 100 % Protein binding : 31% Metabolism : Liver 50-70% Elimination Half Life: 3-7 hr Excretion :kidney and fecal
<b>Pharmacodynamics</b>	Linezolid is, used the treatment of infections caused by aerobic Gram-positive bacteria. The in vitro spectrum of activity of Linezolid also includes certain Gram-negative bacteria and anaerobic bacteria. Susceptible organisms include Methicillin- and vancomycin-resistant staphylococci, vancomycin-resistant Enterococci, penicillin-resistant pneumococcal and anaerobes. Oxazolidinone inhibit protein synthesis by binding at the P site at the ribosomal 50S subunit. Resistance to other protein synthesis inhibitors does not affect oxazolidinone activity, however rare development of oxazolidinone resistance cases, associated with 23S rRNA alterations during treatment have been reported. Linezolid inhibits bacterial protein synthesis through a mechanism of action different from that of other antibacterial agents; therefore, cross-resistance between Linezolid and other classes of antibiotics is unlikely.
<b>Absorption</b>	Linezolid is rapidly and extensively absorbed after oral dosing. Maximum plasma concentrations are reached approximately 1 to 2 hours after dosing, and the absolute bioavailability is approximately 100%
<b>Protein Binding</b>	31% protein binding
<b>Metabolism</b>	Linezolid is primarily metabolized by oxidation of the morpholine ring which results in two inactive ring-opened carboxylic acid metabolites: the amino ethoxyacetic acid metabolite (A), and the hydroxyethyl glycine metabolite
<b>Half Life</b>	4.5-5.5 hours

<b>Side Effects</b>	The most common side effects are diarrhea, nausea, vomiting, headache
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### 3.2. MATERIALS AND METHODS <sup>[12, 13]</sup>

#### A. Selection and Procurement of Active Pharmaceutical Ingredient (API) and excipient:

##### 1. Linezolid:

Linezolid is an antibiotic used for the treatment of infections caused by Gram-positive bacteria. A large meta-analysis of randomized controlled trials found Linezolid to be more effective than glycopeptides antibiotics (such as vancomycin and teicoplanin) and beta-lactam antibiotics in the treatment of skin and soft tissue infections (SSTIs) caused by Gram-positive bacteria,<sup>[20]</sup> and smaller studies appear to confirm its superiority over in the treatment of all serious Gram-positive infections. In the treatment of diabetic foot infections, Linezolid appears to be cheaper and more effective than vancomycin.

##### 2. Preformulation Studies:

Preformulation study relates to pharmaceutical and analytical investigation carried out preceding and supporting formulation development efforts of the dosage form of the drug substance. Preformulation yields basic knowledge necessary to develop suitable formulation. Toxicological use. It gives information needed to define the nature of the drug substance and provide frame work for the drug combination with pharmaceutical excipient in the dosage form. Preformulation testing is the first step in the rational development of dosage form of a drug. It can be defined as an investigation of physical and chemical properties of drug substance, alone and when combined with excipient.

### 3. ANALYTICAL PROFILE

#### A. Determination of analytical wavelength:

##### I. Preparation of standard solution of Linezolid:

Accurately weighed 10 mg of Linezolid was dissolved in 100ml of phosphate buffer solution pH 6.8 (conc.100µg/ml). From this solution 1 ml was pipette out in to 10 ml volumetric flask and volume was made up to with phosphate buffer solution pH 6.8 to make 10µg/ml.

##### II. Scanning solution:

The solution containing 10µg/ml of Linezolid in phosphate buffer pH 6.8 was scanned over the range of 400-200nm against phosphate buffer solution pH 6.8 as blank using UV-Visible Spectrophotometer. The  $\lambda_{max}$  for the pure drug was then determined.

## **B. Calibration curve of Linezolid**

### **I. Preparation of working solution:**

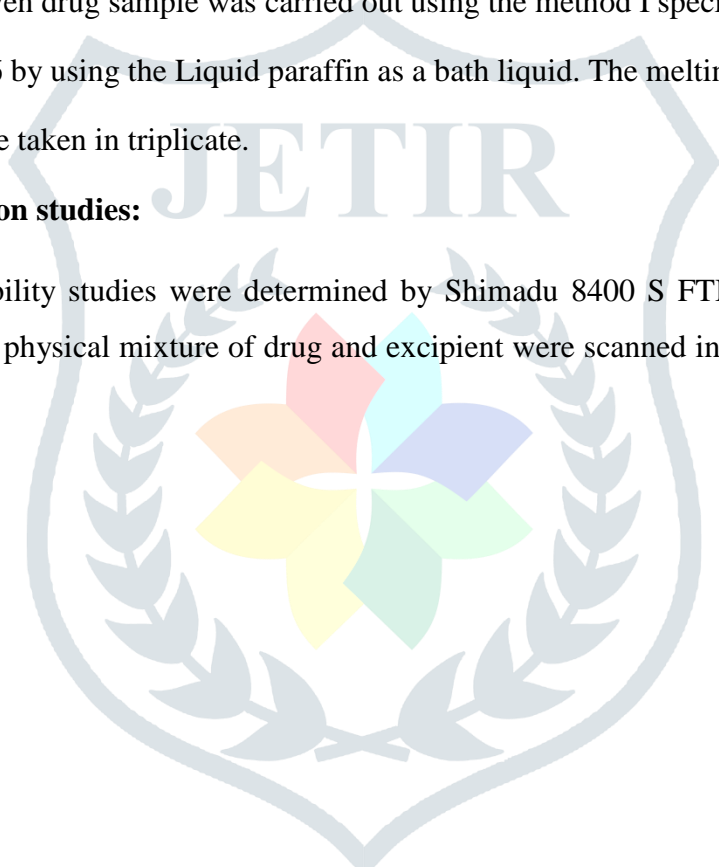
From standard solution, 0.5, 1, 1.5, 2, 2.5 and 3 ml was withdrawn in 10ml volumetric flask and diluted to 10ml with phosphate buffer solution pH6.8 with 0.03% SLS to produce concentration 5, 10, 15, 20, 25 µg/ml respectively. The solution was analyzed by UV-Visible Spectrophotometer (JASCO V-630) at 251nm and results were recorded. The calibration graph was plotted as concentration on x-axis and absorbance on y-axis.

### **II. Melting determination:**

The melting point of the given drug sample was carried out using the method I specified in the Indian Pharmacopoeia 1996 by using the Liquid paraffin as a bath liquid. The melting point was noted and the readings were taken in triplicate.

### **III. Drug-polymer interaction studies:**

The drug excipient compatibility studies were determined by Shimadzu 8400 S FTIR using KBR Pellets of 0.1 mm. sample of pure drug and physical mixture of drug and excipient were scanned in the range between 400-4000  $\text{cm}^{-1}$



## 4. METHODOLOGY: [14, 15, 16]

### 4.1 Preparation of Liposome:

The preparation of liposome with Soybean lecithin was prepared by dried thin film hydration technique using rotary evaporator. Different weight ratios of soya lecithin, cholesterol and Stearic acid were weighed and dissolved in chloroform and methanol mixture (2:1) in 250 ml round bottom flask. A thin film was formed on evaporating organic solvent under vacuum using Rota evaporator at 45-60°C. The dried lipid film was hydrated with 10 ml of phosphate buffer solution (pH 6.8) which containing drug. The dispersion was left undisturbed at room temperature for 2-3 hour to allow complete swelling of the lipid film and hence to obtain vesicular dispersion. This is all shown in [figure 1].

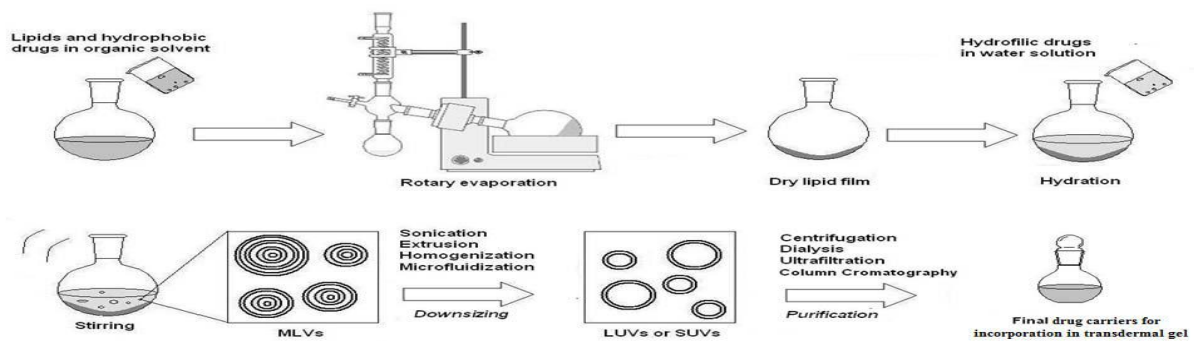


Figure 1: Schematic diagram of preparation of Liposome by thin film hydration Method

### 4.2 Formulation of Liposome:

4.2.

Formulation Code	Drug(mg)	Soya lecithin(mg)	Cholesterol(mg)	Stearic acid(mg)
F1	25	70	30	30
F2	25	100	30	30
F3	25	110	30	30
F4	25	120	30	30
F5	25	150	30	30
F6	25	160	30	30
F7	25	170	30	30

### Preparation of Liposomal Gel:

#### 1).Preparation of Gel:

As a vehicle for incorporation of Linezolid liposome for topical delivery anionic carbopol hydro gel was made. Carbopol 934(1g) was dispersed in dematerialized water (88ml) by stirring at 800rpm for 60 minutes. Then propylene glycol (10ml) was added and the mixture was neutralized by drop wise addition of 10% NaOH. Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 6.8.

## 2) Incorporation of Linezolid Liposome in to the gel

Liposome containing Linezolid were mixed into the 1% (w/w) Carbopol hydro gel by an electrical mixer 25rpm for 5min to get Linezolid liposomal gel.

## 5. PHYSIOCHEMICAL CHARACTERIZATION OF LIPOSOMES:<sup>[17,18,19]</sup>

All the liposomal formulation was evaluated by studying their physicochemical properties like

### 5.1. Vesicle Shape and Size Analysis of Liposome:

Size and shape of the vesicles were determined using optical microscopy and SEM. Determination of surface morphology (roundness, smoothness and formation of aggregates) of Linezolid Liposome with carrier was carried out by scanning electron microscopy (SEM).

### 5.2. Zeta Potential Analysis:

Zeta potential is a physical property which is exhibited by any particle in suspension. It can be used to optimize the formulations of suspensions and emulsions. Knowledge of the zeta potential can reduce the time needed to produce trial formulation. It is also an aid in predicting long-term stability. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate. A value of 25mV (positive or negative) can be taken as the arbitrary value that separates low-charged surfaces from high-charged surfaces. The zeta potential was analyzed by Malvern Zeta sizer

### 5.3. Entrapment Efficiency (EE):

The entrapment efficiency of liposome was estimated by ultracentrifugation method where the liposomal dispersions were centrifuged at 14000 rpm for 30 minutes. The clear supernatant from the resulting solution was diluted appropriately using pH 6.8 phosphate buffer and analyzed for Linezolid spectrophotometrically. The percent of encapsulation efficiency (EE %) was calculated using the following equation.–

$$\text{Total Drug} = \text{EE}\% = \frac{[\text{Total drug}] - [\text{diffused drug}]}{\text{Total Drug}} \times 100$$

### 5.4. In Vitro Drug Release:

Studies were performed for all the formulations. The diffusion cell consisted of a hollow glass cylinder (length 14.6 cm and internal diameter 2.5 cm) made up of borosil glass. One end of the cylinder was covered with egg membrane which was previously soaked in warm water and placed on the receptor compartment. The temperature was maintained at 37°C. Phosphate buffer pH 6.8 was placed in the receptor cell. Samples were

withdrawn at specified time intervals and the medium was replaced with fresh phosphate buffer (pH 6.8). The samples were analyzed for drug using a UV-Vis spectrophotometer at 251 nm.

### 5.5. Short Term Stability Studies:

The stability of a pharmaceutical delivery system may be defined as the capability of a particular formulation, in a specific container. The short-term stability was conducted to monitor physical and chemical stabilities of the liquid form of Linezolid liposomal formulations at 40°C and room temperature for up to three months. The stability parameter, such as Assay was determined as function of the storage time.

## 6. PHYSIOCHEMICAL CHARACTERIZATION OF LIPOSOMAL GEL: [20, 21, 22, 23]

### 6.1. Visual Appearance and pH:

The formulations were observed for the presence of any particular matter. The pH of liposomal topical gels was measured in using digital pH meter. The pH of the liposomal gel was found to be in the range of 6-7.

### 6.2. Viscosity:

Viscosity of the formulations were determined using Brookfield viscometer fitted with S-61 spindle at 10, 20, 50, and 100 rpm

### 6.3. Drug Content:

Drug content was estimated spectrometrically where 100 mg of formulation was taken and dissolved in methanol and filtered. The volume was made to 100 ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 251 nm.

### 6.4. In vitro release studies:

In vitro release studies were carried out using Franz diffusion cell and the temperature was adjusted to  $37\pm 0.5^\circ\text{C}$ . Samples were withdrawn at periodic intervals for 8 hours and replaced with fresh buffer solution to maintain sink conditions. The drug content was analyzed using UV-Visible Spectrophotometer at 251 nm using phosphate buffer (pH 6.8)

### 6.5 Ex Vivo Drug Release Studies:

Ex vivo Studies were carried out using skin of albino rat. Tissue was inserted in the diffusion cell with permeation area of 3.8 cm<sup>2</sup>. Temperature was adjusted to  $37\pm 0.5^\circ\text{C}$ . In situ gel was placed in the donor compartment. At predetermined time intervals, sample was withdrawn and replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed using UV-Visible Spectrophotometer at 251 nm using pH 6.8 phosphate buffers.

## 6.6 Spread ability

A quantity of 0.5 g gel was placed within a circle of 1cm diameter on a pre marked glass slide over which a second glass slide was placed. A weight of 2 g was allowed to rest on the upper glass slide for 1min. The increase in the diameter due to spreading of the gel was noted. Spread ability was then calculated by using the formula:

$$S = M.L / T$$

Where,

S = Spread ability

M = weight attached to upper slide

L = length of spread

T = time taken.

## 6.7. Extrudability

Prepared gel was filled in tube and sealed. 3 Markings were done at interval of 1.5 cm from bottom of tube. The tube was pressed at marking using Pfizer hardness tester with 1 kg/cm<sup>2</sup>, the weight of gel in continuous ribbon expelled is measured for each formulation shown in [figure 2].



**Figure 2: Extrudability Studies Using Pfizer Hardness Tester**

## 6.8. Anti-microbial assay <sup>[24, 25]</sup>

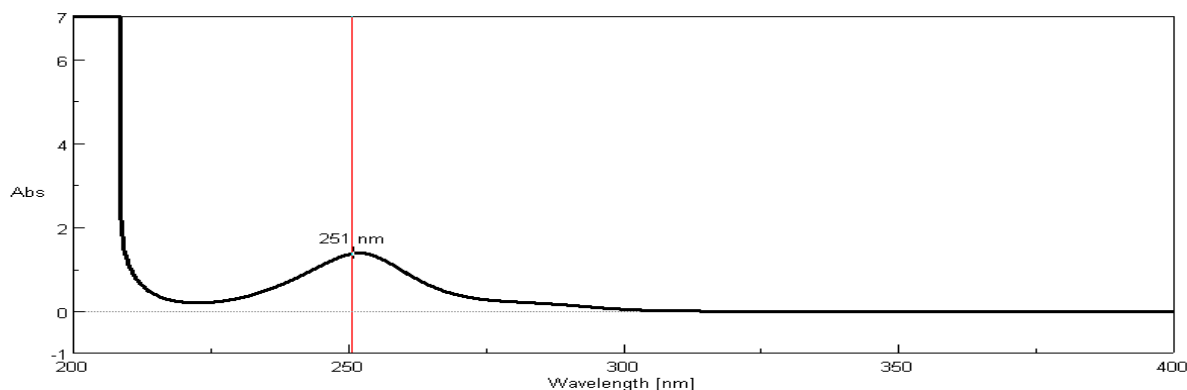
Anti-microbial assay was done by Cup plate method. *S. aureus* broth was prepared by dissolving 0.6 g of *S. aureus* broth in 30 ml of distilled water. Separately, 20 g nutrient agar was dissolved in 200 ml of distilled water. Both broth and agar medium were autoclaved for 15 min at 15 lb pressure. Under aseptic condition, agar media was poured into Petri-dish and distributed evenly in each dish on a level surface and allowed to solidify for half an hour. Bacterial growth of *S. aureus* bacteria was transferred aseptically to the sterilized *S. aureus* broth using sterile nicrom loop and incubate for 24 hrs at bacteriological incubator for complete growth of *S. aureus*. Under aseptic condition *S. aureus* culture was spread over solidified agar plate by using glass spreader. After that cavities were prepared on agar plate using bore and the cavities with standard and sample

solutions and all the Petri dishes were incubated at 37°C for 24 hrs in bacteriological incubator and evaluated for presence of microbial growth.

## 7. RESULT AND DISCUSSION

- **Preformulation Studies:**
- **Analytical profile:**

### A. Determination of analytical wavelength:



**Graph 1- UV spectrum of Linezolid**

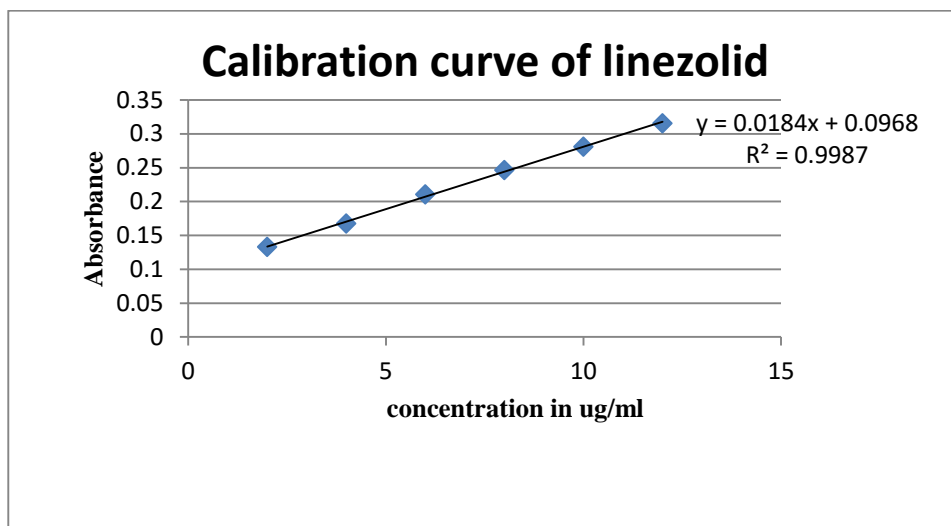
### B. Calibration curve of Linezolid:

The standard calibration curve of Linezolid was obtained by plotting Absorbance vs. Concentration. Table 1 shows the absorbance values of Linezolid the standard curve is shown in graph 2. The standard calibration curve shows the slope of 0.9987 the curve was found to be linear in the concentration range of 5, 10, 15, 20, 25, 30 µg/ml at 251nm. The calculation of drug content, in vitro dissolution study based on this calibration curve parameter shown in table 2.

**Table 1- Calibration Curve of Linezolid:**

Concentration (µg/ml)	Absorbance At 251 nm
0	0
5	0.5248
10	0.6327
15	0.8811
20	1.0637
25	1.3145
30	1.5451





Graph 2- Calibration curve of Linezolid

Sr. No.	Parameters	Values in buffer solution ph 6.8
1	Absorbance maximum ( $\lambda_{max}$ ) in nm	251 nm
2	Slope	0.9987

Table 2- Parameters for calibration curve in pH 6.8 phosphate buffer solution:

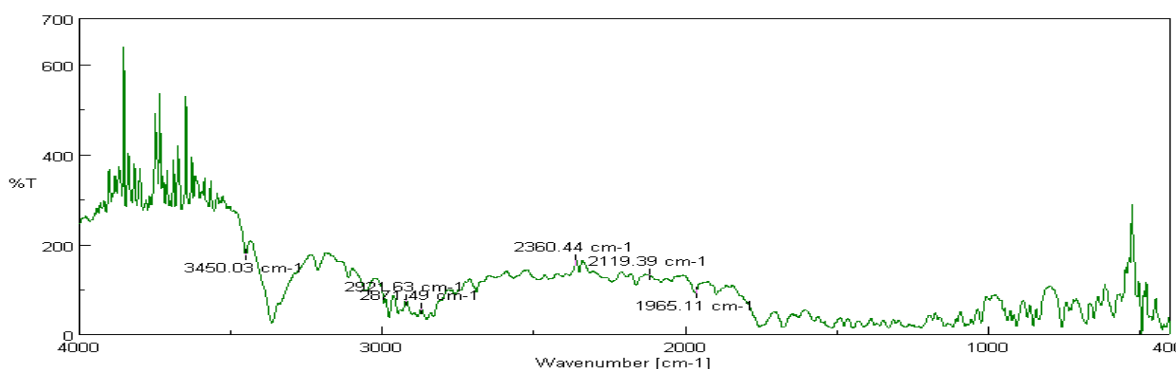
**1. Melting point determination**

Melting point of Linezolid found to be 181.5(practically) as reported in literature, thus Indicating purity of sample.

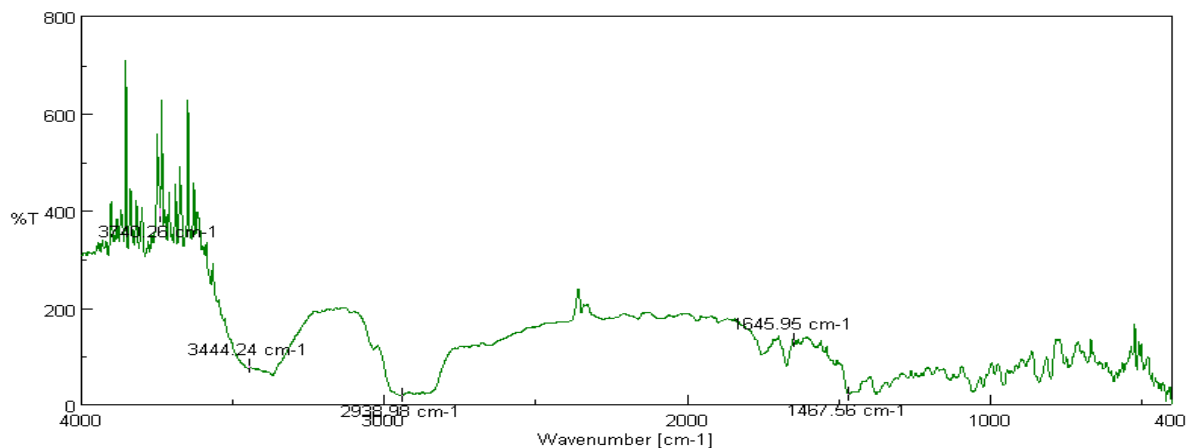
**C. Drug-polymer Interaction Studies**

**1. IR Spectroscopy:**

FTIR spectrum of pure drug and mixture of drug and polymers are shown in Graph 3 and graph 4. From the spectral study, as shown in Table 3 and 4 it was observed that there was no significant change in the peaks of pure drug and drug polymer mixture. Hence, no specific interaction was observed between the drug and the polymers used in the formulations.



Graph 3- IR Spectrum of Linezolid



Graph 4- IR Spectrum of Drug and Excipient

Functional group	Standard frequency (cm <sup>-1</sup> )	Observed frequency (cm <sup>-1</sup> )
N-H Stretching	3100 - 3400	3310
C-H stretching	2820 - 3000	2907
C=O stretching	1800 - 1850	1830
N-H bending	1550-1640	1570

Table 3- Characteristic IR peaks of Linezolid

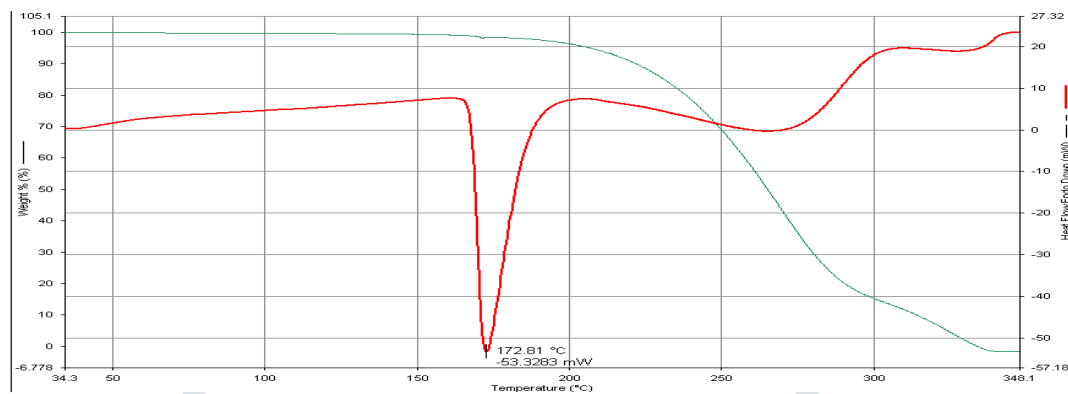
Functional group	Standard frequency (cm <sup>-1</sup> )	Observed frequency (cm <sup>-1</sup> )
N-H Stretching	3100 - 3400	3340
C-H stretching	2820 - 3000	2850
C=O stretching	1800 - 1850	1620
N-H bending		

	1550 – 1640	1590
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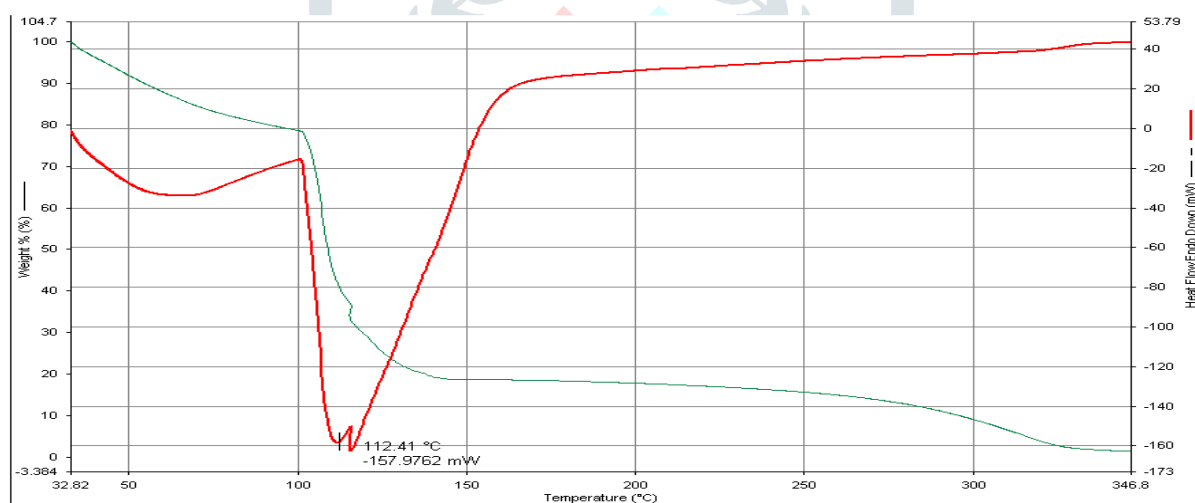
Table 4- Characteristic IR peaks of Linezolid and excipient

**2. Differential Scanning Calorimetry:**

The pre-formulation study of drug-excipient interaction was carried out by DSC, which showed no interactions of the drug and excipient. The melting point of the pure Linezolid was found from the peak of DSC thermo gram at 172.8 °C. The liposome formulation F7 showed a melting point at 112.41. °C that is much lower than all the individual pure components are shown in the graph 5 and graph 6.



Graph 5-DSC curve of pure Linezolid



Graph 6-DSC curve of F1 (Formulation)

❖ **Evaluation of Liposome**

**1. Vesicle Shape and Size of Liposome**

SEM images, microscopic evaluation showed that most of the vesicles were spherical in shape as shown in Fig 3.

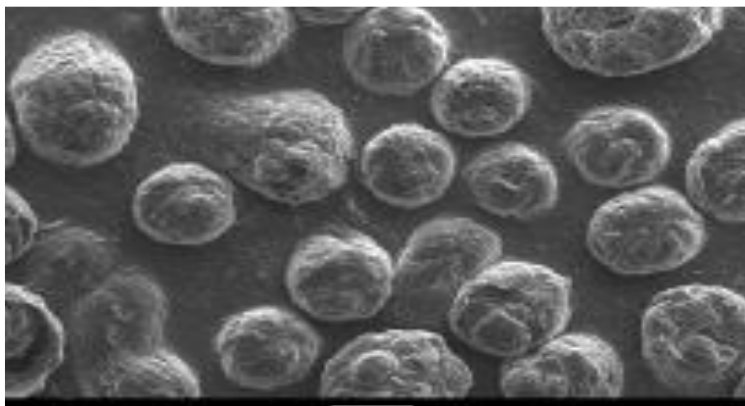
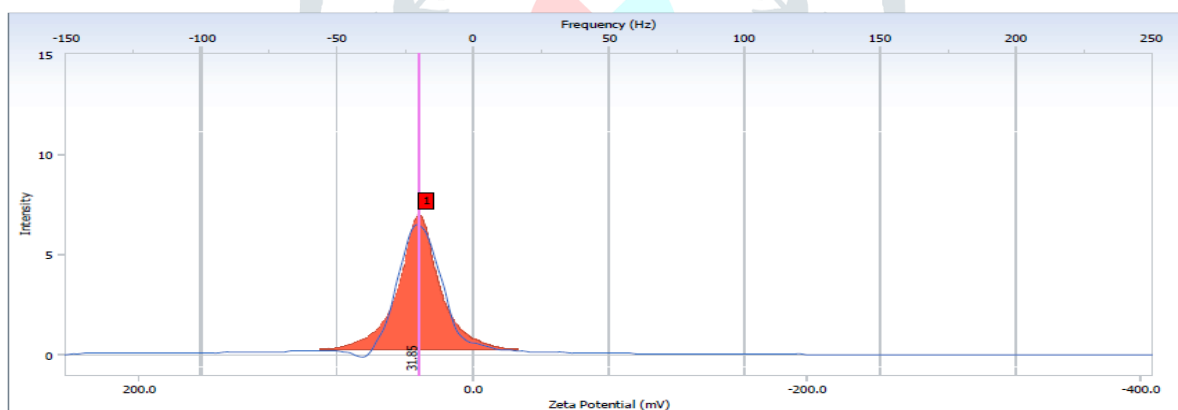


Figure 3: Scanning Electron Microscopic Images of Linezolid liposome

It was observed that most of the vesicles were spherical in shape. The diameter (nm) of liposome was found to be in the range of 300 to 1600 nm. The average size of liposomes was found to be 151nm.

**A .Zeta Potential**

The zeta potential of the liposome was determined using Zeta sizer and the value of the liposome was found to be 31 mV which indicates that liposome were stable are shown in graph 7 and the values for the measurement of zeta potential of Linezolid loaded liposome are shown in the table 5.



Graph 7: zeta potential

Measurement Results

Zeta Potential	: 31.85	(mV)	Doppler shift	: -19.57	(Hz)
Mobility	: 2.484e-004	(cm <sup>2</sup> /Vs)	Base Frequency	: 124.8	(Hz)
Conductivity	: 0.2746	(mS/cm)	Conversion Equation	: Smoluchowski	
Zeta Potential of Cell			Diluent Properties		
Upper Surface	: -229.20	(mV)	Diluent Name	: WATER	
Lower Surface	: 3.61	(mV)	Temperature	: 25.0	(°C)
Cell Condition			Refractive Index	: 1.3328	
Cell Type	: Flow Cell		Viscosity	: 0.8878	(cP)
Avg. Electric Field	: -16.42	(V/cm)	Dielectric Constant	: 78.3	
Avg. Current	: -0.22	(mA)			
Peak Data Table of Distribution Graph					
Peak	Frequency (Hz)	Intensity	Half Width (Hz)	Zeta Potential (mV)	Mobility (cm <sup>2</sup> /Vs)
1	-19.57	7.02	7.29	31.85	2.484e-004

Table 5: Zeta potential of Linezolid loaded liposome

**B. Entrapment Efficiency**

The entrapment efficiency of liposome was estimated by centrifuge (Dragon Laboratory) Instrument. Where the liposomal dispersions were centrifuged at 14000 rpm for 30 minutes.

They were calculated by following equation:

$$EE\% = \frac{[\text{Total drug}] - [\text{diffused drug}]}{[\text{Total drug}]} \times 100$$

### C. Percentage entrapment efficiency of Linezolid in liposome

Formulation Batch	%Entrapment efficiency
F1	73.67 ± 0.22%
F2	78.51 ± 0.24%
F3	78.57 ± 0.28%
F4	81.83 ± 0.26%
F5	85.96 ± 0.24%
F6	87.50 ± 0.28%
F7	88.20 ± 0.24%

Percentage entrapment efficiency of Linezolid in liposome was found to be in the range of 73 – 88% as shown in Table 6. The entrapment efficiency was found to be higher for the formulation F7 (88.20%) as shown in above chart.

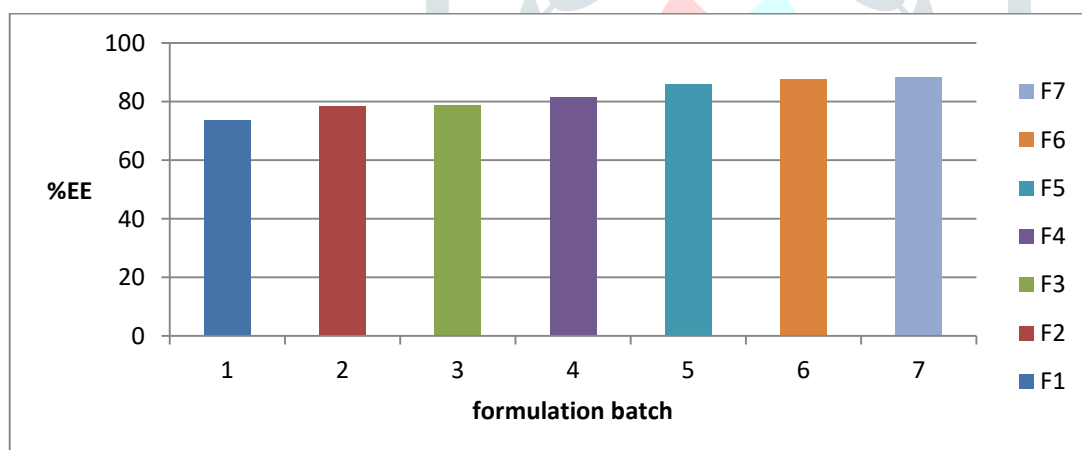


Table 6: Percentage entrapment efficiency of Linezolid in liposome

### D. In Vitro Drug Release of liposome:

In vitro release studies were carried out using Franz diffusion cell in figure 4. The Samples were withdrawn at periodic intervals for 8 hours and replaced with fresh buffer solution to maintain sink conditions. The drug content was analyzed using UV-Visible Spectrophotometer at 251 nm using phosphate buffer (pH 6.8) as blank

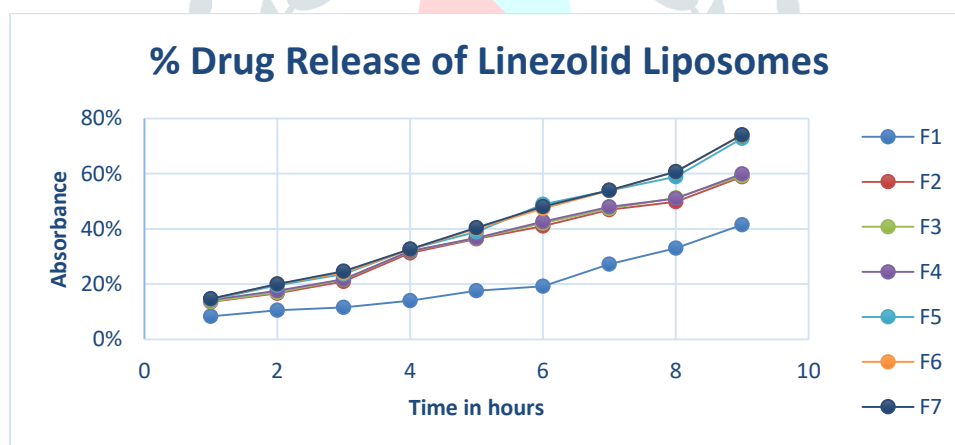


Figure 4: Franz Diffusion Apparatus.

Sr.no	%Drug Release of Liposomes						
	F1	F2	F3	F4	F5	F6	F7
1	8.36%	13.60%	13.62%	14.20%	14.40%	14.60%	14.7%
2	10.52%	16.64%	17.04%	17.58%	19.64%	20.04%	20.08%
3	11.60%	21.02%	21.68%	21.83%	23.66%	24.08%	24.68%
4	14.04%	31.32%	31.85%	32.04%	32.63%	32.56%	32.67%
5	17.60%	36.36%	36.60%	36.79%	38.92%	40.08%	40.41%
6	19.20%	41.04%	42.11%	42.69%	48.88%	47.44%	48.08%
7	27.20%	46.92%	47.42%	47.96%	53.88%	53.88%	53.98%
8	33.04%	49.82%	51.25%	51.03%	58.92%	60.72%	60.78%
9	41.48%	58.92%	59.26%	59.98%	72.76%	74%	74.04%

Table.7: Percentage Drug Release of Liposome

The percentage of drug release from various liposomal formulations is shown in graph 8. The experimental studies showed that the rate of drug release depends on the concentrations of the contents of the formulations. Formulation in table 7 shows FL 7 showed higher drug release than other formulations. Hence, it was chosen to be formulated as liposomal topical gel.



Graph 8: Percentage Drug Release of Liposomes

## E. Evaluation of Topical Liposomal Gel

### 1. Visual Appearance

All formulations are, white in color, odorless, semi-solid in nature and had smooth appearance.

### 2. PH

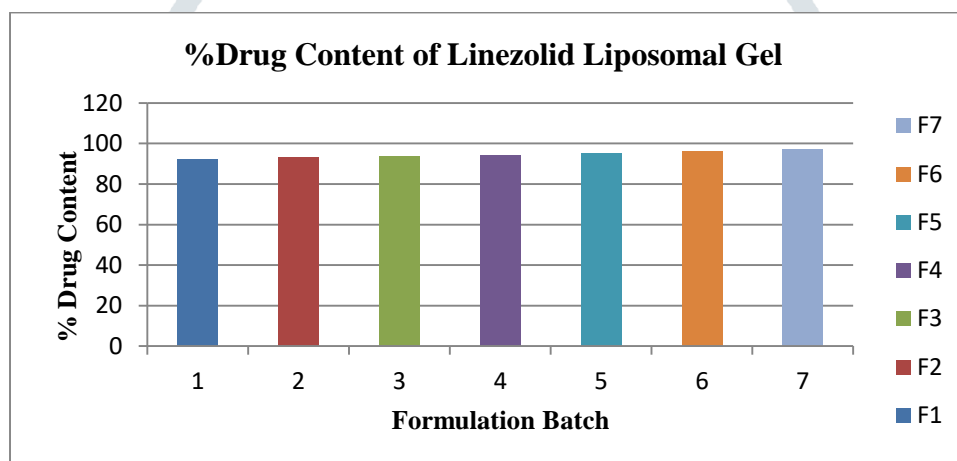
pH of all formulations was in the range between 6.1 to 6.7. The formulation batch (F7) pH was found to be 6.3.

### 3. Drug Content

The solutions were analyzed for drug content spectrophotometrically at 251nm. All the formulation batch exhibited fairly uniform drug content. The drug content of all formulations was in the range of 92 to 98% as shown in Table 8 and the formulation drug content shown in the graph 9.

Sr. No.	Formulation Batch	% Drug Content
1	F1	92 ± 0.26 %
2	F2	93.2 ± 0.28 %
3	F3	93.7 ± 0.24 %
4	F4	93.9 ± 0.22 %
5	F5	95 ± 0.26 %
6	F6	96.2 ± 0.24 %
7	F7	97.1 ± 0.32 %

Table 8. Percentage Drug Content of Liposomal Gel



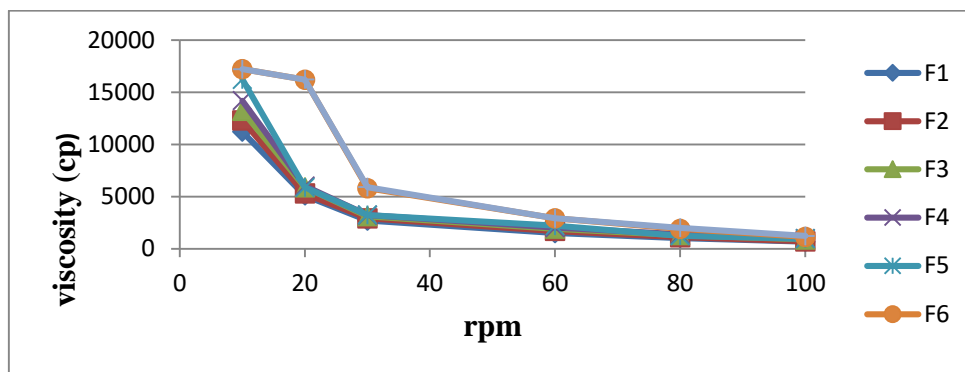
Graph 9: %Drug Content of Linezolid Liposomal Gel

#### 4. Viscosity:

Viscosity of the formulations were determined using Brookfield viscometer at spindle no. 61.in 10,20,30,60,100,rpm.The viscosity of the all gel formulations ranged from 625- 17223cps shown Table 9. The viscosity of the formulations decreased on increasing the shear rate shown in the graph 10.

Sr no.	RPM	Viscosity (cps)						
		F1	F2	F3	F4	F5	F6	F7
1	10	11219	12321	13219	14201	16240	17220	17223
2	20	5120	5309	5907	6020	5904	16211	16229
3	30	2712	2920	3120	3231	3240	5804	5921
4	60	1509	1711	1931	2039	2220	2921	2927
5	80	1029	1109	1307	1397	1249	1911	2012
6	100	685	709	902	977	972	1149	1231

Table 9: Viscosity of Liposomal Gel



Graph 10: Viscosity of Linezolid Liposomal Gel

### 5. In Vitro Drug Release of Linezolid Liposomal Gel:

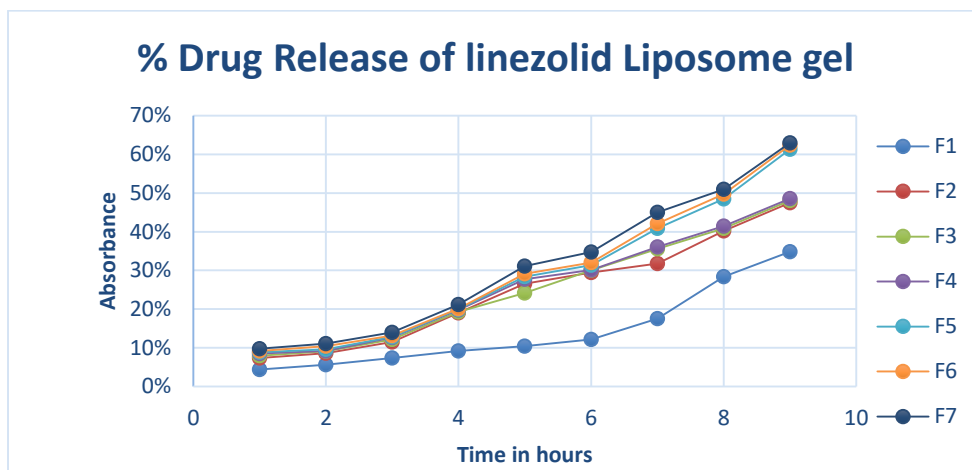
In vitro release studies were carried out using Franz diffusion cell. The Samples were withdrawn at periodic intervals for 8 hours and replaced with fresh buffer solution to maintain sink conditions. The drug content was analyzed using UV-Visible Spectrophotometer at 251 nm using phosphate buffer (pH 6.8) as blank. This is all shown in the table 10.

Time in hrs	%Drug Release of Linezolid Liposomal Gel						
	F1	F2	F3	F4	F5	F6	F7
1	4.36%	7.4%	7.96%	8.55%	8.90%	9.2%	9.8%
2	5.6%	8.6%	9.15%	9.16%	9.60%	10.40%	11.13%
3	7.36%	11.52%	12.21%	12.70%	12.84%	13.20%	14%
4	9.2%	19.04%	19.32%	19.81%	19.95%	20.08%	21.16%
5	10.4%	26.6%	24.17%	27.69%	28.42%	29.12%	31.08%
6	12.2%	29.48%	30.06%	30.14%	31.36%	32.02%	34.78%
7	17.56%	31.80%	35.58%	36.04%	40.88%	42.08%	44.98%
8	28.4 %	40.24%	40.90%	41.42%	48.48%	49.72%	50.92%
9	34.8%	47.52%	48.08%	48.59%	61.32%	62.40%	62.97%

Table 10: Percentage Drug Release of Linezolid Liposomal Gel

The percentage of drug release from various liposomal formulations is shown in graph 10. The experimental studies showed that the rate of drug release depends on the concentrations of the contents of the formulations. Formulation FL 7 showed higher drug release than other formulations. Hence, it was chosen to be formulated as liposomal topical gel.





Graph 10: Percentage Drug Release of Linezolid Liposomal Gel

**6. Stability Studies of Optimized Formulation:-**

Stability studies were performed as per ICH guidelines at accelerated condition of temperature of  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and humidity of  $60\% \text{ RH} \pm 5\% \text{ RH}$  for three months and evaluated for- size, zeta Potential ,entrapment efficiency, gel characteristics and In vitro permeation. The drug content, pH and gelling capacity were determined for every 30 days for 3 months. It was observed that there was no change in the physical appearance of the formulation. The drug content was analyzed and there was marginal difference between the formulations kept at different temperatures as shown in Table 11. Liposomal topical gel formulations retained good stability throughout the study.

Storage Conditions	pH			
	Initial	1 month	2 month	3 month
$25 \pm 2^{\circ}\text{C}/$ $60 \pm 5\% \text{ RH}$	6.2	6.4	6.5	6.7

Storage Conditions	Drug content			
	Initial	1 month	2 month	3 month
$25 \pm 2^{\circ}\text{C}/$ $60 \pm 5\% \text{ RH}$	97.1	96.8	96.2	95.7

Table 11: Stability data of Linezolid Liposomal Gel

## 7. Spreadability

A quantity of 0.5 g gel was placed within a circle of 1cm diameter on a pre marked glass slide over which a second glass slide was placed. A weight of 2 g was allowed to rest on the upper glass slide for 1min. The increase in the diameter due to spreading of the gel was noted. Spread ability was then calculated by using the formula shown in table 12:

$$S = M.L / T$$

Where,

S = Spread ability

M = weight attached to upper slide

L = length of spread

T = time taken.

Sr. No.	Formulation Batch	Spreadability (gm.cm/ sec)
1	F1	1.12
2	F2	1.18
3	F3	1.21
4	F4	1.24
5	F5	1.28
6	F6	1.30
7	F7	1.38

Table 12. Spreadability of Linezolid Liposomal Gel.

The value of spread ability was found to be between the ranges of 1.12 to 1.38 g.cm/sec as (Table 12). The highest spread ability was observed for formulation (F7).

## 8. Extrudability

Prepared gel was filled in tube and sealed. 3 Markings were done at interval of 1.5 cm from bottom of tube. The tube was pressed at marking using Pfizer hardness tester with 1 kg/cm<sup>2</sup>, the weight of gel in continuous ribbon expelled is measured for each formulation shown in figure 5.



Figure 5: Extrudability Studies Using Pfizer Hardness Tester

Sr. No	Formulation Batch	Extrudability (gm)		
		Press - 1	Press - 2	Press - 3
1	F1	2.53	1.30	1.02
2	F2	2.60	1.45	1.05
3	F3	2.75	1.65	1.07
4	F4	2.88	1.93	1.09
5	F5	3.22	2.01	1.19
6	F6	3.44	2.09	1.63
7	F7	3.91	2.17	1.95

Table 13: Extrudability of Linezolid Liposomal Gel

From table 13, based on evaluation of Extrudability of all the formulations, of Linezolid Liposomal Gel was observed in range 1.95 -3.91gm. They have good Extrudability and ease to applications.

### 9. Anti-microbial assay

Anti-microbial assay was done by Cup plate method shown in figure 6. S.aureus broth was prepared by dissolving 0.6 g of S. aureus broth in 30 ml of distilled water. Separately, 20 g nutrient agar was dissolved in 200 ml of distilled water. Both broth and agar medium were autoclaved for 15 min at 15 lb pressure. Under aseptic condition, agar media was poured into Petri-dish and distributed evenly in each dish on at level surface and allowed to solidify for half an hour. Bacterial growth of S. aureus bacteria was transferred aseptically to the sterilized S. aureus broth using sterile nicrom loop and incubate for 24 hrs at bacteriological incubator for complete growth of S. aureus. Under aseptic condition S. aureus culture was spread over solidified agar plate by using glass spreader. After that cavities were prepared on agar plate using bore and the cavities with standard and sample solutions and all the Petri dishes were incubated at 37°C for 24 hrs in bacteriological incubator and evaluated for presence of microbial growth.

Sr.No	Formulation Batch	Zone of Inhibition
1	F1	7mm
2	F2	9mm
3	F3	10mm
4	F4	11mm
5	F5	13mm
6	F6	14mm
7	F7	16mm
8	Pure Linezolid Drug	17mm

**Table 14: Anti-microbial assay of Linezolid Liposomal Gel**



**Figure 6: Anti Microbial Assay of Linezolid Liposomal Gel**

Zone of inhibition of the formulations were found in the range of 7-16 mm (table 14). Batch (F1) showed lower antimicrobial action (7mm) and Batch (F7) Showed higher antimicrobial action which was comparable to Linezolid solution (17mm).

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