## MATERIALS AND METHODS

### Table-2: Chemicals:

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Manufactured by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carvedilol</td>
<td>Chandra Laboratories (Hyderabad).</td>
</tr>
<tr>
<td>Soya lecithin</td>
<td>Research lab Fine chem. Industries (Mumbai)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Jiangsu Huaxi International Trade Co.Ltd (CHINA)</td>
</tr>
<tr>
<td>Carbopol-934</td>
<td>Research Lab fine chem. Industries (Mumbai)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Virat lab (Mumbai).</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>Research lab Fine chem. Industries (Mumbai)</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>Research Lab fine chem. Industries (Mumbai)</td>
</tr>
<tr>
<td>Ultrapure water</td>
<td>Cortex laboratories (Hyderabad)</td>
</tr>
</tbody>
</table>
Equipments:

**Table-3: Instruments and company**

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electronic weighing balance</td>
<td>Shimadzu corporation (JAPAN).</td>
</tr>
<tr>
<td>UV-Spectrophotometer</td>
<td>Shimadzu 1800(JAPAN).</td>
</tr>
<tr>
<td>Magnetic stirrer</td>
<td>REM electro technique limited. Vasai (INDIA)</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>Allwyn (INDIA).</td>
</tr>
<tr>
<td>Sonicator</td>
<td>SISCO Scientific Instruments sales Corporation, Thana, Mumbai.</td>
</tr>
<tr>
<td>pH meter</td>
<td>EI</td>
</tr>
<tr>
<td>Scanning electron microscope</td>
<td>Shimadzu corporation (JAPAN).</td>
</tr>
<tr>
<td>FTIR</td>
<td>Shimadzu corporation (JAPAN).</td>
</tr>
<tr>
<td>Humidity chamber</td>
<td>SISCO Scientific Instruments sales Corporation, Thana, Mumbai.</td>
</tr>
<tr>
<td>Hot Air Oven</td>
<td>Lawrence and Mayo Pvt Ltd. Mumbai.</td>
</tr>
<tr>
<td>Blood Pressure Apparatus</td>
<td>Inc Santa Barbara. U.S.A.</td>
</tr>
</tbody>
</table>
DRUG PROFILE

CARVEDILOL:

Synonyms : Carvedilolum [Latin]

Molecular formula : C24 H26N2O4

IUPAC Name : [3-(9H-carbazol-4-yloxy)-2-hydroxypropyl] [2-(2-Methoxyphenoxy) ethyl] amine

Molecular Weight : 406.8

Structure :

![Figure 16: Structure of Carvedilol](image)

Appearance : White crystalline powder

Therapeutic Category:

- Antihypertensive agent,
- Adrenergic Agents,
- Adrenergic beta Antagonists,
- Vasodilator Agent,
- Adrenergic alpha-Antagonists.
Physical properties:

- It is off white fine crystalline powder.
- Solubility: Readily soluble in Di-methyl Sulf-oxide, Methanol, Methylene chloride and in Iso-propanol, 95% Ethanol it is sparingly soluble whereas in Ethyl Ether it is slightly soluble and in insoluble in gastric pH, intestinal fluids and water.

Chemical properties:

Clinical use:

Along with ACE inhibitors it is used in the treatment of CHF. It has extra morbidity and mortality benefits in the treatment of severe CHF.

PHARMACOLOGY

The Carvedilol medicament is an enantiomer mixture. Both the isomers have α-adrenergic receptors blocking properties and the levorotatory β-adrenergic receptor blocking property.

Mechanism of Action:

The β-adrenergic receptor blocking ability produces the following pharmacological actions namely reduction in heart rate myocardial contractility oxygen demand and systemic vascular resistance. Carvedilol along with its metabolites present Ca$^{2+}$ ATPase in sarcoplasmic reticulum. Hence they are in the management of CHF they are beneficial.

Pharmacokinetics:

Absorption:

It gets rapidly retained orally. After one hour of Carvedilol administration the bioavailability plasma level increases by 25%. The plasma levels of dextrorotatory form of Carvedilol increases 2-4 times higher than the levorotatory form due to stereo selective first pass digestion system.
**Distribution:**

More noteworthy is the point that 98% of Carvedilol is retained in plasma proteins, principally egg whites as it is highly lipophilic. The correct volume is 2 L/kg and increases in patient assessed by the liver.

**Metabolism:**

After oral administration the drug enters into the first pass metabolism Carvedilol metabolized into its metabolites

**Excretion:**

Disposal is chiefly via bile and the drug. Small protons are excreted by kidneys. In old patients the T max and AUC series are found to be increased. Carvedilol plasma levels are more or less of higher in the elderly constructed with adolescent subjects.

**Melting point:**

114-115\(^0\)C.

**Clearance:**

500-700 ml/min.

**Half life:**

An elimination half life of 6-10hrs had been reported.

**Distribution volume:**

115 L

**Adverse effects:**

- Dizziness
- Hypotension
- Fatigue
- Hyperglycemia
- Bradycardia
- Weight gain
• Diarrhoea
• Asthenia.

Protein binding:

Albumin is the plasma protein to which 98% of Carvedilol is bounded.

Bio-availability:

Absolute bioavailability for 3mg dose is 40%

Storage:

Store in a tightly closed container at room temperature of 20°C to 25°C (68°F to 77°F) and away from excess heat and moisture.

Dosage:

The following are the dosages of Carvedilol 3-125mg, 6.25mg, 12.5mg and 25mg.

DRUG INTERACTIONS:

Effects of other drugs on Carvedilol via the Cytochrome p450 system:

Interactions demonstration:

The average of the penultimate disposal half-life for Carvedilol was observed to be similar to that in sound subjects.

Since Carvedilol experiences considerable oxidative digestion system consideration may be needed in patients getting inducers (e.g. Cimetidine increased the area under the curve of Carvedilol by approximately 30%. However change in curve was observed. Around 16% increase in the AUC of Carvedilol was achieved by 300ml of grape juice.

Theoretical interactions:

Interactions of CYP2D6 inhibitors via quinidine, Fluoxetine, paroxetine and propafenone were found to spike the blood levels.
Drug-drug Interactions:

Digoxin:

15% plasma concentration of Digoxin is increased by Carvedilol. Thus expanded observing of Digoxin is prescribed when starting, altering or stopping Carvedilol.

Cyclosporine:

An unobtrusive increment in the average minimum cyclosporine was observed after Carvedilol treatment in renal transplant patients. Cyclosporine bindings should be checked after Carvedilol ingestions.

Clonidine: Administration of Clonidine with beta-blocking drugs may increase the circulatory strain and heart rate bringing down infarcts. Clonidine should not be taken along with the β-blockers. In the first phase β-blocker treatment should be given later on Clonidine can be given.

Calcium channel blockers:

Like the β-blockers, Ca⁺ channel blockers treatment must be monitored carefully under the close supervision and monitoring of pulse and ECG.

Anti-arrhythmic drugs:

Anti-Arrhythmic drugs (Beta-blockers) are to be used with caution as stated above for the different classes of drugs.

Laboratory tests:

Carvedilol does not influence research centre tests.

INDICATIONS:

In the management of CHF C

Carvedilol is used along with Vasodilators, ACE inhibitors, Digoxin and Diuretics.
CONTRAINDICATIONS:

- In the hypersensitive, allergic reactions and disorders like asthma, Carvedilol will worsen the condition.
- It also worsens the condition of brady-cardia to severe sinus brady-cardia.
- Shock.
- Known excessive touchiness to Carvedilol.
- Hepatic disease.
EXCIPIENT PROFILE

LECITHIN

Non-proprietary Names:

USP-NF: Lecithin

Synonyms:

• LSC 5050
• LSC 6040;
• Mixed soya bean phosphatides;
• Ovolecithin;
• Phosal 53 MCT;
• Phospholipon 100 H;
• Soya bean phospholipids;
• Sternpur;
• Vegetable lecithin.

Chemical Name and CAS Registry Number, Composition:

Lecithin 8002-43-5 1, 2-diacyl-sn-glycero-3-phosphocholine (trivial chemical name, Phosphatidylcholine). Depending on the source of the lecithin the purity and properties varies. In Egg lecithin 24% Phosphatidylethanolamine and 69% Phosphatidylcholine is present, whereas in soybean lecithin 19% Phosphatidylinositol, 22% Phosphatidylethanolamine and 21% phosphatidylcholine is present.
The structure of Lecithin (and consequently additionally its physical properties) change immensely relying on the origin of the lecithin and the level of filtration. R1 and R2 are unsaturated fats, which may be distinctive or indistinguishable. In the b-structure, the phosphorus containing gathering and the R2 gathering trade positions.

**Functional Category:**

- Emollient,
- Emulsifying executor,
- Solubilising operator.

**Description:**

Lecithins differ incredibly in their physical structure, from thick semi fluids to powders, contingent on the free unsaturated fat substance. They might likewise shift in colour from tan to light yellow, contingent on whether they are dyed or unbleached or on the level of immaculateness. When they are presented to air, quick oxidation happens, likewise bringing about a dim yellow or tan colour.

**Typical Properties:**

*Saponification value:* 196
Solubility:

Lecithins are dissolvable in aliphatic and fragrant hydrocarbons, halogenated hydrocarbons, mineral oil, and unsaturated fats. They are essentially insoluble in frosty vegetable and creature oils, polar solvents, and water. At the point when blended with water, nonetheless, Lecithin’s hydrate to structure emulsions.

Stability and Storage Conditions:

Lecithins decay at compelling pH. At the point when warmed, lecithins oxidize, obscure, and disintegrate. Temperatures of 160–180°C will result in corruption within a day time. At higher temperature like 108°C partitions will be formed, it is done on liquid or waxy lecithin evaluations. All Lecithin evaluations ought to be put away in overall shut compartments secured from light and oxidation. Refined strong Lecithins ought to be put away in firmly shut compartments at subfreezing temperatures.

Applications in Pharmaceutical Formulation and Technology:

Lecithins are used as components of wide mixture of pharmaceutical applications

- Lecithins are predominantly utilized as a part of pharmaceutical items as scattering, emulsifying, and balancing out operators, and are incorporated in intramuscular and intravenous infusions, parenteral sustenance definitions, and topical items, for example, creams and balms.

- Lecithins are likewise utilized as a part of suppository bases, to diminish the weakness of suppositories, and have been researched for their assimilation improving properties in an intranasal insulin definition. Lecithins are additionally regularly utilized as a part of enteral and parenteral nourishment plans.

- Liposomes in which lecithin are incorporated as a segment of the bilayer has been utilized to epitomize drug substances; their potential as novel conveyance frameworks has been Utilitarian Category.
PROPYLENE GLYCOL

Nonproprietary Names:

United State Pharmacopeia: Propylene Glycol

Synonyms:

- Propylenglycolum;
- Propane-1, 2-diol;
- Methyl glycol;
- Methyl ethylene glycol;
- E1520; 2-hydroxypropanol;
- 1, 2-Dihydroxypropane.

Molecular Weight and Empirical Formula:

C₃H₈O₂ 76.09

CAS Registry Number and Chemical Name:

1, 2-Propanediol [57-55-6] (-) 1, 2-Propanediol [4254-14-2] (+) 1, 2-Propanediol [4254-15-3]

Structural Formula:

![Figure 18: Structure of Propylene glycol](image)

Functional Category:

- Antimicrobial additive
- Disinfectant
- Humectant
• Plasticizer
• Dissolvable
• Settling executor
• Cosolvent (water-miscible)

Pharmaceutical Formulation and Technology Applications

• In the pharmaceutical formulations like parenteral etc; the Propylene glycol is utilized as dissolving agent, solvent, solute and additive. It is having a great dispersible and solubility activity than glycerin. Thus it is used to dissolve various pharmaceutical ingredients like most alkaloids, Vitamins (A & D) Barbiturates, Sulfa medications, Phenols, Corticosteroids and numerous neighborhood soporifics.
• It is also used as a preservative, like Ethanol it is a germ-free agent, like Glycerin it is effective against molds.
• As a plasticizer it is used like fluid film-covering definitions.
• Propylene glycol is likewise utilized as a part of beauty care products and as topical applications.

Table 4: Propylene glycol applications in pharmaceutical formulation and technology

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Concentration (%)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerosol solutions</td>
<td>10-30</td>
<td>Solvent or co solvent</td>
</tr>
<tr>
<td>Solutions &amp; semisolids</td>
<td>15-30</td>
<td>Preservative</td>
</tr>
<tr>
<td>Topical</td>
<td>≈ 15</td>
<td>Humectants</td>
</tr>
<tr>
<td>Topical</td>
<td>5-80</td>
<td></td>
</tr>
<tr>
<td>Parenterals</td>
<td>10-60</td>
<td></td>
</tr>
<tr>
<td>Oral solutions</td>
<td>10-25</td>
<td></td>
</tr>
</tbody>
</table>

Physical Characters:

Similar to glycerine it is having sweet slightly acrid taste, practically odorless liquid, viscous, colourless and clear.
Physical Properties:

Density 1.038 g/cm³ at 200⁰C

Boiling point 188⁰C

Solubility:

It is completely soluble in (CH₃)₂CO, water, Glycerine, Ethanol (95%) and Chloroform; Partially soluble in ether at the ratio of 1:6 and insoluble in settled oil and mineral oils but it disintegrates in the vital oil.

Storage Conditions and Stability:

Propylene glycol is stable at low temperatures and in a closed shut holder conditions. If the temperature is elevated or if it is placed in open then similar to that of acetic acid, pyruvic acid, lactic acid and propionaldehyde, it gets oxidized, liberating ascent. If it is blended with water or glycerine and 95% ethanol it gets stabilized. By performing autoclave sterilization its aqueous products can be done.
CHOLESTEROL

Non-proprietary Names:

USP-NF: Cholesterol

Synonyms:

- Cholesterolum,
- Cholesterin.

Molecular Weight and Empirical Formula:

\[ C_{27}H_{46}O \ 386.67 \]

CAS Registry Number and Chemical Name:

Cholest-5-en-3ß-ol [57-88-5]

Structural Formula:

![Figure 19: Structure of Cholesterol](image)

Category:

- Emulsifying agent,
- Emollient.

Applications:

- It is used as an emollient in the topical applications and balms; it is also used as emulsifying agent at conc. 0.3–5.0% w/w in cosmetics products.
• It is a lipid that is found in all the higher class of living organisms, it is stored in all the tissues and body parts and acts as a source of energy and controls the vital functions along with the thermo-regulation.

• **Description:**

  It is very nearly scentless, powder, needles, silvery flyers or granules. Colour varies from white to pale yellow on exposure to air, heat and light colour changes to faint yellow.

  **Melting point:**

  \[147^0C – 150^0C\]

  **Boiling point:**

  \[360^0C (some decomposition)\]

  **Dielectric constant:**

  \[D^{20} = 5.41\]

  **Density:**

  \[1.052 g/cm^3 \text{ anhydrous form.}\]

**Storage Conditions and Stability**

It is a stable compound that is meant to be stored in a closed container in a dark place, away from light.

**Solubility:**
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility at 20°c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>Soluble</td>
</tr>
<tr>
<td>Methanol</td>
<td>1 in 294 at 0°c</td>
</tr>
<tr>
<td></td>
<td>1 in 153 at 20°c</td>
</tr>
<tr>
<td></td>
<td>1 in 53 at 40°c</td>
</tr>
<tr>
<td></td>
<td>1 in 34 at 50°c</td>
</tr>
<tr>
<td></td>
<td>1 in 23 at 60°c</td>
</tr>
<tr>
<td></td>
<td>1 in</td>
</tr>
<tr>
<td>Isopropyl myristate</td>
<td>1 in 19</td>
</tr>
<tr>
<td>Hexane</td>
<td>1 in 52</td>
</tr>
<tr>
<td>Ether</td>
<td>1 in 2.8</td>
</tr>
<tr>
<td>Ethanol [95%]</td>
<td>1 in 78 (slowly)</td>
</tr>
<tr>
<td></td>
<td>1 in 3.6 at 80°c</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1 in 147 at 0°c</td>
</tr>
<tr>
<td></td>
<td>1 in 78 at 20°c</td>
</tr>
<tr>
<td></td>
<td>1 in 29 at 40°c</td>
</tr>
<tr>
<td></td>
<td>1 in 19 at 50°c</td>
</tr>
<tr>
<td></td>
<td>1 in 13 at 60°c</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1 in 4.5</td>
</tr>
<tr>
<td>Benzene</td>
<td>1 in 7</td>
</tr>
</tbody>
</table>
CARBOPOL

Non-proprietary Names:

USP-NF: Carbomer

Synonyms:

- Acrypol;
- Tego Carbomer,
- Pemulen;
- Carboxyvinyl polymer;
- Polyacrylic acid;
- Carboxy polymethylene;
- Carbopol;
- Carbomera;
- Acrylic acid polymer;
- Acritamer.

CAS Registry Number and Chemical Name:

Carbomer [9003-01-4]

Molecular Weight and Empirical Formula:

They are the compounds with carboxylic acid up to 52%- 68% cross linked either with pentaerythritol allyl ethers or sucrose allyl ether. They are very high molecular weight compounds weighing around 7 X 10^5 to 4 X 10^9.
Applications:

- They are employed as a rheological property modifier in the semisolid or liquid dosage forms meant for tropical, rectal, vaginal and ophthalmic route of applications.
- Carbomer polymers have additionally been explored in the readiness of managed discharge lattice globules, as they inhibit the enzymatic degradation by protease enzyme. And as they have peptide they can be employed in the preparation of mucoadhesive, intranasal and cervical patch that adhere to the site and release the drug in controlled manner.
- An increase in the cross link in carbomers will enhance the controlled drug release but it cannot be influenced by the thickness of polymers.
- In the solid dosage forms they are employed to release the drug in controlled dose.
- It is used as a emulsifier in oil-in-water emulsion as it has consistency expanding support for microspheres.

Functional Category:

- Bioadhesive material;
- Controlled-discharge executor;
- Emulsifying operator;
- Emulsion stabilizer;
- Rheology modifier;
- Settling operator;
- Suspending operator;
- Tablet cover.

Applications:
They additionally utilized within beautifying agents.

Table 6: Carbopol applications in pharmaceutical formulation and technology

<table>
<thead>
<tr>
<th>Use</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled release agent</td>
<td>5.0-30.0</td>
</tr>
<tr>
<td>Tablet binder</td>
<td>0.75-3.0</td>
</tr>
<tr>
<td>Suspending agent</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>Gelling agent</td>
<td>0.5-2.0</td>
</tr>
<tr>
<td>Emulsifying agent</td>
<td>0.1-0.5</td>
</tr>
</tbody>
</table>

Physical characters:

A granular Carbomer is also available (Carbopol 71G). They are having characteristic odour, hygroscopic powders, acidic, fluffy and white-coloured.

Melting point:

When heated at 260°C. For 30 min. it decomposes.

Glass transition temperature:

100–105°C

Dissociation constant:

pKa = 6.0 ± 0.5

Tapped Density:

0.4 g/cm³ (granular) and 0.3 g/cm³ (powder);

Bulk Density:

0.4 g/cm³ (granular) and 0.2 g/cm³ (powder);
Moisture content:

2% w/w On the other hand, Carbomer are hygroscopic and normal balance dampness
content at 258°c and half relative stickiness is 8–10% w/w.

Solubility:

As they are micro-gels with 3D cross-interface they swell to noteworthy degree but
don’t disintegrate.

Dynamic (Viscosity):

Acidic colloidal are produced when Carbomers are dispersed in water to deliver very
thick gels. In the blended water first powder Carbomer must be dispersed (to avoid the
agglomerates), which can be killed by the expansion of a base.

Storage Conditions:

Exposure to extreme temperatures can bring about staining and decreased solidness.
Complete deterioration happens with warming for 30 minutes at 260°c. Triethanolamine is
broadly utilized as a part of topical pharmaceutical details, basically in the shaping of
emulsions.
TRIETHANOLAMINE

Non-proprietary Names:

USP-NF: Trolamine

Synonyms:

Trolaminum; tris (hydroxyethyl) amine; trihydroxytriethylamine; triethylolamine; Tealan; TEA.

Molecular Weight and Empirical Formula:

\[ C_6H_{15}NO_3 \] 149.19

CAS Registry Number and Chemical Names:

2, 2’, 2’’-Nitrilotriethanol [102-71-6]

Category:

Emulsifying agent, alkalizing agent.

Structure:

![Structure of Triethanolamine](image)

Figure 21: Structure of Triethanolamine

Applications:

- In the emulsion formulation and tropical applications Triethanolamine is employed.

- It is also employed in the sun screen preparations and also for the formation of salts for the injections.
• It is also utilized like a self to solubulise substances for injectable solutions, topical analagsic and surfactants, textile specialties, waxes, polishes, herbicides, toiletries, buffers, solvents, humectants etc.

Physical characters:

It is a viscous liquid with odour of ammonia, coloured colourless to pale yellow and clear liquid. Bases namely 2- amino ethanol (mono ethanolamine); 2, 2’- iminobisethanol (di ethanol amine) and 2, 2’, 2”-Nitrilotriethanol together make Triethanolamine.

Other physical properties:

Viscosity:

590 mPas (590 cps) at 30°C

Moisture content:

0.09%

Melting point:

20–21°C

Hygroscopicity:

Very hygroscopic.

Freezing point:

21.6°C

Boiling point:

335°C

Acidity/alkalinity:

pH = 10.5 (0.1 N solution)

Solubility:
Table-7: Triethanolamine Solubility

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility at 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Miscible</td>
</tr>
<tr>
<td>Methanol</td>
<td>Miscible</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>1 in 63</td>
</tr>
<tr>
<td>Carbon tetra chloride</td>
<td>Miscible</td>
</tr>
<tr>
<td>Benzene</td>
<td>1 in 24</td>
</tr>
<tr>
<td>Acetone</td>
<td>Miscible</td>
</tr>
</tbody>
</table>

**Storage Conditions and Stability:**

When exposed to light and air Triethanolamine will become brown. Store in an air tight container. Protect from light and keep it cool and dry.
ETHOSOMES:

The drugs which have a property of low penetration via biological membrane can be delivered by this new carrier system known as Ethosomes. Ethosomes are a modified form of liposomes. Ethosomes constitute soft vesicles of lipid containing phospholipids, water and alcohol (isopropyl alcohol and ethanol) in a comparatively higher concentration. They have a size which ranges from tens of nano meters to microns.\cite{35-37}

The permeation of Ethosomes via skin layers is more rapid and they possess transdermal flux in a higher range. The delivery of active agents is enhanced by the ethosomes and it chiefly comprises of phospholipids (Phosphatidylserine, Phosphatidyl acid, Phosphatidylcholine) water and higher concentration of alcohol. Ethosomes are unique to the presence of higher concentration of ethanol in it, whose nature is to disturb the bilayer organisation. Thus when put in a vesicle; the vesicle gains the ability to get penetrated into the skin layers of S.corneum. Tightly packed lipid membrane is due to the higher ethanol concentration where stability does not change. It allows even structures which are more malleable and thus distribution of the drug is improved.\cite{38}

Ethosomes comprise of phospholipids, water and ethanol. Phosphatidyl glycerol (PPG) Phosphatidyl Inositol, Phosphatidyl ethanol amine PE, hydrogenated PC and Phosphatidyl choline PC are varied chemical structure containing phospholipids. The range of non-aqueous phase may vary from 22% to 70% and the type of alcohol may vary either isopropyl alcohol or ethanol. For characterization studies fluorescent probes of amphiplic
nature such as Rhodamine-123, D-289, 6-carboxy fluorescence, Fluorescence Isothiocyanate (FITC) are usually added to the ethosomes.\textsuperscript{39-40}

**Mechanism:**

Ethosomal skin penetration depth can be assessed by confocal laser scanning microscopy (CLSM). Different fluorescent probes can be employed to study the penetration of skin. Physico-fluorescent probes with chemical properties like Carotene C, Rhodamine red, Rhodamine 6G, Rhodamine B can be enclosed inside the Ethosomal vesicles\textsuperscript{41-42} are employed. The temperature for transition of the vesicular systems of lipid is known in the form of softness of vesicle and it is influenced by the drug and the concentration of Ethanol. The determination of stability storage of Ethosomal systems can be done by comparison of the average size, shape, capacity of entrapment by the vesicles at varied conditions of storage. Depending on the different studies conducted on stability, researchers have suggested that the suitable storage condition for Ethosomes are refrigeration condition which is (4-8)\textdegree C where as at higher temperatures there is a possibility of vesicular lipids to get degraded , the structural integrity of the vesicles is lost and also the contents which are entrapped also get leaked.
Advantages of drug delivery of Ethosomes:

- The permeation of drug through the skin in trans-dermal route is enhanced.
- Larger molecules like protein molecules, peptides can also be delivered by it.
- The formulation consists of raw material which is nontoxic.
- Patient compliance is observed highly in Ethosomes due to its ability to be administered in semisolid form (gel or cream).
- The Ethosomes are available for immediate commercialization, they are even found to be passive and non-invasive\(^{43}\).
- These Ethosomal drug delivery systems have applications in varied fields like Pharmaceutical, Cosmetic and Veterinary.
- It is a simple method of drug delivery system when compared to the other complicated methods like phonophoresis and Iontophoresis.
• Ethosomes enhances the penetration of trans-dermal and dermal drug delivery.
• Larger molecular proteins and peptides can be delivered at larger dose by the help of Ethosomes.
• Ethosomal components have been approved for cosmetic and pharmaceutical purpose.
• Ethosomes components toxicological profiles are documented, so the technology of low risk profile has no development risk of large scale.
• As Ethosomes are administered in semisolid forms both in gel or in cream form so complication in patients are more, where as Photophoresis and Iontophoresis are very complicated and have poor patient compliance.
• For the products with proprietary technology there is high market attractiveness. For the production of Ethosomes the manufacture is relatively very simple without complicated technical investments.

Limitations of Ethosomes:

• The yield is poor.\textsuperscript{44}
• If there is ineffective shell locking then the coalescence of ethosomes is seen and on transferring them into water they fall apart.\textsuperscript{45}
• When it is transferred from organic media to water media there is a loss of product seen.

1.6.2.1 Method of preparation of Ethosomes:

The following are the formulation methods for ethosomes:

Hot method:

At $40\degree$C phospholipids is first heated in the water, till the colloid is obtained. Propylene glycol and ethanol are mixed in a separate vessel then it is mixed with water phase at $40\degree$C. Then either in ethanol or water drug is dissolved based on their solubility. Then by using either the extrusion method or probe sonication method the ethosomal formulation's vesicle size can be modified to the desired extent\textsuperscript{40,41}.
Cold method:

In a covered vessel dissolve the drug by stirring vigorously in ethanol, lipid materials and phospholipids. Before heating the mixture up to $30^\circ\text{C}$ on water bath, mix polyglycol or propylene glycol, later on add preheated water. Then by using either the extrusion method or probe sonication method the ethosomal formulation's vesicle size can be modified to the desired extent\textsuperscript{46, 47}.

Figure 24: HOT METHOD
Classic mechanical dispersion method:

In a round bottomed flask, a mixture of CCl₄: soya phosphatidylcholine (1:3) methanol is dissolved. By using the rotary vacuum evaporator the removal of organic solvents is done over the temperature of lipid transition film of lipid is formed which is very thin like flakes on the wall. Leaving contents overnight under vacuum solvent traces can be removed. Hydration is done by rotating the flask at a temperature which is suitable with different concentrations of mixture of hydroethanolic solution containing drug.⁴⁸,⁴⁹

Classic method:

In a water bath ethanol is poured with the drug and phospholipids dissolved in it and then it are heated to a temperature of 30°C ± 1°C. Add lipid mixture with water in a fine stream which is distilled double times, stir with constant rate of 700rpm. The resultant suspension of vesicle is subjected to homogenization by passing via a membrane made up of polycarbonate by using a hand extruded for a time period of three cycles⁵⁰.

The Ethosomes technology advantages are

- Commercially and scientifically proven to be feasible,
- As it is patent internationally protected.
- Approved and safe materials are used for its preparation.
- It can be used for the large group of drugs that has different chemical nature
- It can also be used for various dosage forms
- Non-invasive and Passive
- Permeation of drug can be enhanced
- It is a known published technology and is also established one.

For the protection from the external climatic conditions, temperature loss and water loss from the tissues a principal barrier has been evolved, which is also a route of drug administration to the systemic circulation. In comparison with the atmospheric water the human body water concentration is much more that is 50M, which drives the water from the body to the atmosphere that is to be prevented. Thus skin acts as a barrier interface for water efflux and also prevent the influx of chemicals and other harmful agents. It also helps in regulation of body temperature (Barry, 1983). For a large variety of drugs TDDS is a convenient route of administration with a surface area for absorption (1-2 m²) and challenging scientist over 25 years for the formulation preparation.

Transdermal applications are a simple and non-invasive when compared to the other routes (Band-Aid applications is a simple patch application). A wide range of TDDS are formulated and are available for example for hypogonadism oestradiol a HRT, for Smoking cessation Nicotine, For pain pentanyl, for CVS disease nitro-glycerine and Clonidine, and for motion sickness scopolamine are available (Benson 2005). TDDS is more recommended for many formations due to its better patient compliance and effectiveness leading for formulations of many drugs like CVS drugs, hormone preparations and pain relievers. TDDS when applied on to the skin it permits drug absorption from skin to the tissue and circulation because of its discrete dosage form and self contained though few drugs are delivered through this route it holds 12% of worldwide market revenues of US $3B shared by 7% Japan, 32% Europe and 56% USA and further it is estimated to grow 12% annually as per (Front Line Strategic Consulting Inc) due to its low compliance and simplicity it is also preferred in geriatric and paediatric patients and also to avoid the daily medication, in patients who cannot swallow. Over the oral route of administration it has several advantages like dose required is low as in oral the drug degradation by enzymatic and hepatic first pass metabolism takes place. Which was overcome by the IV route for skipping the first pass metabolism of drug,
prolonged and effective drug delivery but it has its limitation as IV route cannot be given in all cases where as TDDS can be given.

Trans dermal drug delivery system (TDDS) is free from these disadvantages in addition it provides the benefits of using the drug with shorter biological half life, low dose is sufficient as drug loss due to metabolism is not there, easy terminal of drug in case of ADR. In past decade dramatic transformation were made for the simply designed transdermal patch that include the drug concentration, size , covering area, drug therapeutic level, penetration enhancers. First generation of Patches were plastic pieces with adhesive edges were dipped in drug dissolved in alcohol and used as patches which lead to number of skin problems and gave a negative impact (Morro, 2004). Later generation is still in use – “drug in the adhesive” where the drug is suspended in the adhesive which acts as glue to attach on the body, this model has reduced skin irritant and even eliminated in many cases. But stickiness of adhesive is directly affected by drug concentration in adhesive, thus to increase the dose the patches size should be increased or else frequency of reapplying the patches was needed.

This problem was solved in the third generation using acrylic reservoir for holding the drug and semisolid suspension of microscopic cells with concentrated drug and Silicon adhesive. In the fourth generation bolus dosing is introduced by increase the skin permeability, by the use of drug delivery enhancers like electroporatic methods, lasers, sonophoresis, micro needles and chemicals including gels, ultrasound and Iontophoresis, (Morrow, 2004). World widely they are used for the treatment of many disorders related to CVS like heart failure and hypertension and also approved by Food and Drug Administration. With less ADR among β- adrenergic blockers, Nebivolol is tolerated drug that on long-term therapy improves exercise capacity, clinical endpoints of death, left ventricular function and cardiovascular hospital admissions of stable heart failure patients (Veverka et al., 2007). The present research work deals with the formulation and evaluation of the Nebivolol matrix type transdermal drug delivery system using synthetic polymers i.e. Eudragit RL100 or Eudragit RS 100 & HPMC, which offers low cost, high availability, biodegradable, biocompatible, non-toxic and ease of chemical modification. It also provides controlled release characteristic of the controlled drug delivery system (Sannino, et al., 2009). The TDS route provides sustained plasma profile for a prolonged duration of time that reduces the drug plasma levels fluctuations. As it is a non-invasive technique with it improved patient compliance and also easy to remove the patch from the site for terminates the action
ANATOMY, PHYSIOLOGY AND FUNCTION OF THE HUMAN SKIN

Human skin is the largest organ covering the body, protecting from noxious chemicals and microorganisms regulating water loss and heat of the body due to its uniquely engineered structure. As it is largest organ that keeps outside out and inside in, self repairing easily accessible organ provides multiple sites for drug administration for both systemic and local actions. It covers an area of 1.7 m² and possess body mass of 10% in average person who’s complexity can be examined at different levels. According to mathematical treatments skin is a simple physical barrier where as its complexity define it as a series of barriers that transport drug in the tissue through pores. While examining membrane basic structures and functions complexity degrees is seen. Membrane metabolic activity limits transdermal drug delivery In some extremely conditions. Due to immunological responses optimal proven formulation during in vitro studies may not be clinically used.

PROPERTIES THAT INFLUENCE TRANSDERMAL DRUG DELIVERY

Based on three factors as skin, drug and vehicles effective TDDS can be formulated. Physicochemical factors and biological factors are two classes affecting TDDS (Zhou and Wu, 1997; Sharma et al., 2011).

1.3.1 Biological factors

i) Skin condition:

Penetration is promoted by the solvents like Methanol, Chloroform, Alkalis and Acids by damaging the skin cells. Intact skin acts as a good barrier but the diseased condition of patient alters the skin conditions.

ii) Skin age:

Drug penetration is affected by the age of skin; toxins are well penetrated in young skin than older. Thus age of skin is one of the factors of in TDDS.
iii) **Blood supply:**

Transdermal absorption can be affected by peripheral circulation changes.

**Iv) Regional skin site:**

Factors affect penetrations significantly are appendages density, stratum corneum nature and thickness of skin.

v) **Skin metabolism:**

Drug penetration efficacy depends on skin metabolism as it metabolizes some drugs, chemical carcinogens, hormones and steroids.

vi) **Species differences:**

The skin penetration is affected by keratinisation, density of appendages and thickness of skin that from vary species to species.

**1.3.2 Physicochemical factors:**

i) **Skin hydration:**

Skin is hydrates easily with water due to which its permeability increases significantly. Thus humectants are used in transdermal delivery.

ii) **Temperature and pH:**

When temperature falls the decrease in diffusion coefficient occurs. And when the temperature variation occurs drug permeation increase ten folds. Based on the pH and pKa or pKb value weak acids and weak bases dissociate. The drug concentration in skin can be determined by proportion of unionized drug. Thus, temperature and pH are important factors affecting drug penetration.

iii) **Diffusion coefficient:**

Diffusion coefficient of drug At a constant temperature depends on diffusion medium properties, properties of drug and interaction between drug and diffusion medium. Depending on drug diffusion coefficient penetration of drug takes place.
iv) **Drug concentration:**

If the drug concentration is more across the barrier then the gradient concentration will be higher. i.e., flux is proportional to the gradient concentration across the barrier.

v) **Partition coefficient:**

Intercellular route is the pathway for stratum corneum traverse for the molecules with highly lipophilic (log K > 3) and intermediate partition coefficient (log K 1 to 3), they also have ability to partition out of stratum corneum into the viable epidermal aqueous tissue. Transcellular route is predominant in more hydrophilic molecules (log K < 1).

vi) **Molecular size and shape:**

Drug absorption is inversely proportional to the Drug size i.e., smaller is the molecular size greater is the penetration rate vice-versa. Flux of drug material through the human skin is also a major factor that depends on the drug size. Thus it is said that transdermal flux and molecular weight of the molecule are inversely proportional as the molecular size and weight are approximately same. Where the molecular weight (100-500 Dalton) of therapeutic agents is desirable for transdermal delivery.

vii) **Other factors:**

There are many other factors apart from the above discussed factors that alter the drug penetration through skin such as Drug binding, drug and tissue interaction that is altered by some factors like Vanderwals forces and hydrogen bonding. This intern affects the flux of drug across tissue based on permeant.

**Pathological disorders**

The skin disorders, pathology and their symptoms are described detailed in many textbooks where in many of the disorders the functions of barrier are compromised, that improves by the treatment till the tissue is restored where the disorder is treated completely and the decrease in the drug penetration occurs due to restoration of tissue.

**ERUPTIONS:**

Due to the eruption of surface of the skin in disorders the *Stratum corneum* compromises the barrier properties allowing easy passage of drugs and other materials through skin, similarly the water loss is increased in case of erupted skin. Above the basal
layer two to three layers of keratinocytes are increased due to mitosis in psoriatic Plaques condition. There are many therapies that exist for treatment, including mitotic activity inhibitors [e.g., psoralen-UVA (PUVA) treatment]. Loss of skin barrier is facilitates the drugs targeting to the desired site and minimizing side effects (Anigbogu et al., 1996). Flat-topped papules and intensely itchy are characteristics of Lichenoid eruptions where in lichen planus thickening of granular epidermal layer and in dermis at basement membrane lymphocytes are found suggesting it to be a autoimmune disease. In Eczema (to boil over in Greek) blistering occurs which is a non-infectious eruptive.

In the patient with strong genetic predisposition like asthma or allergic rhinitis etc, epidermis chronic inflammation called Atopic dermatitis occurs, in which stratum corneum is the barrier highly compromised leading to increased water loss of 10-fold (Ogawa and Yoshiike, 1992; Aalto-Korte and Turpeinen, 1993). Direct irritant contact on dermatitis like allergens, previous sensitized substances and other toxic agents like detergents, solvents and chemical substances causes Irritant dermatitis which shows an onset of action of drug 4-12 hr after exposure. Irritant dermatitis was induced in vivo model using sodium lauryl sulphate for the assessment of various drugs (Wilhelm et al., 1991).

**Infections**

Bacteria, yeast and other micro-organism when breach the tissue (contain microbial flora) and the intact skin that is a effective barrier against the microorganisms ingress leads to infections. The commonly infecting microorganism accumulates around the hair follicles like propionibacteria, corynebacteria, micrococci and staphylococcal species (e.g., *Staphylococcus epidermidis*). For example at forearm 60 per cm² of micrococci where as at axillae it count to 500,000 per cm², such a huge number of micro-organism may definitely alter the drug absorption by metabolizing it prior to absorption.

Most common skin disorders are caused by the staphylococcal species (*S. aureus, S. pyogenes*). Necrotising fasciitis is a severe infection of *S.pyogenes* causing minor trauma i.e., an ill-defined Erythema that turns to necrotic if not removed surgically and treated with systemic antibiotics it may become fatal. Other less common Gram-positive bacterial infections like Mycobacterium *tuberculosis* leads to tuberculosis also arise condition called lupus vulgaris which is characterized by face and neck plaques red/brown colour. Benign cutaneous tumours called as Warts (verrucae) are caused by the human papilloma virus which is transmitted by direct contact.
In the viral infections the stratum granulosum keratinocytes gets vacuolated and epidermis hyperkeratosis leading to the thickening and formation of eruption takes place. Tropical applications are used for the hand warts treatment and for genital warts cryotherapy is used. Where tropical application demonstrate the targeting of salicylic acid on the viral particles Lawson et al., 1998). Self-limiting vesicular eruptions caused by the infection of Herpes simplex (cold sores) is also topically treated. Secondary bacterial infections caused by Herpes zoster (shingles) can be problematic even though it is a self limiting. The most common and may turn to severe infections are fungal infections. Keratinised tissues is been targeted by the Dermatophyte infections and damage to skin integrity is dependent on the severity infection. Serious impairment is observed in the cases of necrotising fasciitis.

**Ichthyosis**

Dry and scaly skin with disorders of keratinisation and epidermal differentiation are the Characteristic of Ichthyoses. Excessively dry and scaly skin with increased in thickness of stratum corneum and stratum granulosum is seen in ichthyotic even though the compromised barrier integrity is seen in patients of ichthyotic (Lavrijsen et al., 1993).

**Formulation approaches**

Colloidal carrier’s usage enhances Penetration of formulation. Entrapment active molecules into the skin are to be done by the use of submicron sized particles which include carriers like solid-lipid nanoparticles, nano-emulsions and liposomes (Fig.11). Localized effect is reported in the most cite, whereby the carriers accumulate in upper skin layers or stratum corneum. Generally, it is unexpected that the penetration of colloidal carrier’s viable skin may occur. However, the carrier’s effectiveness is still under debate.
Transferosomes, a new type of liposomes that consist of cholesterol, phospholipids and sodium cholate (surfactant molecules) has been introduced. It is claimed by the inventors that the transferosomes are ultra deformable and can squeeze the pores till one-tenth of its diameter. Transferosomes of 200 to 300 nm-sized are claimed to penetrate intact skin. Under \textit{in vivo} conditions penetration of colloidal particles works best when hydrated. This gives a scope of enhance skin penetration for preparing new formulation of micro-emulsions that contains oil, water and amphiphilic compounds (surfactant and co-surfactant) that yield thermodynamically stable, isotropic and transparent stable liquid. They may be water continuous, oil continuous or bio-continuously. Particle size of the dispersed phase is the main difference between the macro and micro-emulsion. In case of micro-emulsion there is an order of magnitude (10-200nm) where as in conventional emulsions (1-20 µm). Solubility of both hydrophobic and hydrophilic components, thermodynamic stability, and optical transparency are the micro-emulsions typical properties. Increased drug concentration in the micro-emulsion enhanced the penetration that provides a large concentration gradient from vehicle to skin. The oil and surfactants in the micro-emulsions interact with the rigid lipid bilayer structure to act as chemical enhancer (Schmalfuss et al., 1997).

1.7 Prodrug Approaches:

From the investigations of pro-drug approach in dermal and transdermal delivery of drug enhancement in drug delivery with unfavourable partition coefficients was absorbed.
To increase the partition coefficient the addition of promote is with pro-drug is a strategically approach that enhances the transport and solubility of drug in the stratum corneum. Using S6- acyloxymethyl and that of 5-fluorouracil, the poor permeability of 6-mercaptopurine is increased by 240 times, by forming N-acyl derivatives skin permeability was increased up to 25 times of a polar drug (Beall & Sloan, 2002). For other drugs like NSAIDS and beta blockes were also investigated for the pro-drug approach (Davaran et al., 2003; Doh et al., 2003). In the human skin the charged drug don’t readily penetrate thus lipophilic ion pairs has been studied for increasing drug penetration into stratum corneum.

**Liposomes:**

A liposome is a small vesicle or bubble which comprises the same material as that of the membrane of the cell. These can be used for delivering the anti-cancer drugs as well as drugs for treating other diseases by filling them in the liposomes.

The British haematologist Dr. Alec D Bingham FRS described about liposomes in 1961 in the Babraham institute in Cambridge which was published in 1964. This was discovered by Bang ham and R.W. Horne while testing the electron microscope of the institute by the addition of negative stain to the phospholipids which were dry. It resembled to the plasma lemma and the first true evidence that the cell membrane is a lipid structure with bi layers was given by the microscopic pictures.

The derivation of the word liposome has been done from the two words of Greek are 'Lipos' which means 'fat' and 'soma' which means 'body'. In the liposomes there is an internal aqueous volume which is covered by a membrane of lipid bi layer giving it a structure of concentric bleeder vesicles. These membranes are usually comprised of phospholipids, which constitute of a head group of hydrophilic nature and a tail group of hydrophobic nature. As the head is hydrophilic in nature it gets attracted to water and the tail which is hydrophobic in nature gets repelled by water, it is made of a long hydrocarbon chain.
Figure 27: Scheme of a liposome formed by phospholipids in an aqueous solution.

The stable membranes are naturally found to be made up of bi layers of phospholipids. When water is present the head group gets attracted to water and they make up to form a surface which faces the water where as the tail group is repelled by it and they form a surface away from the water. So in a cell we can observe that one layer will have its heads facing outer side of the cell i.e. attracted to the environment's water where as the other layer will be facing the inner side of the cell attracted to the water which is in the cell. Both the hydrocarbon layers face each other to give an appearance of bilayers.  

If there is a disruption of the phospholipids membrane then they have the capability to reform into spheres, which is small when compared to a normal cell either as monolayer’s or bilayer where the bilayer structures are known as liposomes and the monolayer structures are known as micelles. The plasma membrane mainly constitutes of phospholipids for example phosphatidylcholine and phosphatidylethanolamine.

These phospholipids are amphiphlic whose nature is hydrophobic consist of tails that are hydrocarbons. Plasma membrane contains moisture (water) on this interior as well as the exterior surface thus the hydrophobic tails face each other in the phospholipids. Liposomes can be made up of phospholipids which are derived naturally with lipid chains which are mixed for example egg phosphatidylethanolamine or DOPE (dioleoyl phosphatidylethanolamine) which is a pure surfactant component.

Generally liposomes constitute an aqueous solution in the core but by definition it is not defined so. The liposomes which do not have aqueous material are known as micelles however to encompass an environment of aqueous nature reverse micelles can be made.
**Pros:**

Few of the pros are as follows:

- The targeting to tumour tissues is done in a selectively passive manner.
- The therapeutic index and efficacy is increased.
- Enhanced stability through encapsulation.
- The agents which are encapsulated have a reduced toxicity.
- The pharmacokinetic effects are improved (Enhanced circulation life times, reduced elimination).
- For the achievement of active ligands there is flexibility in coupling with site specific ligands.
- Site avoidance effect.\(^{33}\)

**Varieties of liposomes:**

The liposomes can be classified based upon various considerations namely:

- Structural parameters.
- Preparation method.
- Applications and composition.

**Characterizations of Liposomes:**

**Visualization:**

Liposomes can be visualized by using transmission electron microscopy (TEM) and microscopic electronic Scanning (SEM)\(^ {51}\).

**Zeta potential and Vesicle size:**

Vesicle size and the zeta potential can be determined using photon correlation spectroscopy (PCS) and dynamic light scattering (DLS)\(^ {52}\).
Entrapment efficiency:

By the use of ultra centrifugation technique the efficiency of entrapment of the drug by the Liposomes can be measured\textsuperscript{53}.

Transition temperature:

Using differential scanning calorimeter the determination of transition temperature of the lipid systems of particles can be done\textsuperscript{54}.

Measurement of Surface tension activity:

Surface tension activity of the drug in aqueous solution can be found by using Du Nouy ring tensiometer following ring method\textsuperscript{55}.

Vesicle stability:

The stability can be determined by assessing size and structure of the particles. TEM is used to measure structure changes and DLS is used to measure mean size\textsuperscript{56}.

Penetration and permeation studies:

The depth of penetration of Liposomes can be visualised by (CLSM) Confocal-laser-scanning-microscopy\textsuperscript{57}.

As ethosomes are malleable vesicles, soft and potential carriers for drug transportation interest in the formulation and development is increasing tremendously. Active drug permeation can be enhanced by tailoring due to efficacy, safety, and simplicity in their preparation of ethosomes. When compared to hydro-alcoholic solution and liposomes, ethosomes are more efficiently deliver the drug to skin. Peptides, proteins, cationic drugs and hydrophilic drugs can be encapsulated in the ethosomes. For the novel therapies development ethosomal carriers provided new opportunities as well as opened challenges which can be beneficial for total health care potential of universe.
<table>
<thead>
<tr>
<th><strong>Drug</strong></th>
<th><strong>Results</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium glycyrrhizinate</td>
<td>Anti-inflammatory activity is enhanced by providing sustained release due to deposition of drug that is due to the improved penetration.</td>
</tr>
<tr>
<td>Azelaic acid</td>
<td>- Release of drug is prolonged.</td>
</tr>
<tr>
<td>Anti-HIV agents</td>
<td>- The activity is enhanced by 2-3 folds due to Enhanced drug flux through transdermal Decreased toxicity of drug Exended action of drug Donot effect the normal skin histology</td>
</tr>
<tr>
<td>Lamivudine Zidovudine</td>
<td></td>
</tr>
<tr>
<td>Bacitracin</td>
<td>- Bioavailability enhanced increased deposition of drug in dermis Deliver of drug intracellular is improved</td>
</tr>
<tr>
<td>Cannabidol</td>
<td>- Biological activity is improved</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>- Action time of drug is increased</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>- Deposition of drug in the skin is enhanced</td>
</tr>
<tr>
<td>DNA</td>
<td>- Genes expression is better Target selectivity of dermal cells</td>
</tr>
<tr>
<td>Trihexyphenidyl hydrochloride</td>
<td>- At a very low dose biologically active of drug can be attained. Compliance related to patient improved</td>
</tr>
</tbody>
</table>
### FACTORS AFFECTING CHARACTERS OF ETHOSOMES

Ethosomes consist of ethanol (10%), phosphatidyl choline (0.5%) and drug. Concentration of ethanol and phospholipids are the factors which affect the characters of ethosomes e.g. vesicular size, entrapment efficiency and dermal delivery. Their effects are as follows:

**Ethanol:**

Using DLS method (dynamic light scattering) the size distribution concentration of ethosomes can be estimated. With the elevation of conc. above 50% of ethanol the vesicle size is found to be decreased. At 50% ethanol the smallest vesicles were formed whereas at 10% ethanol largest vesicles were formed.

From the studies and data available it show that the efficiency of drug entrapment also dependent on concentration of ethanol, at higher conc. (ethanol >30% w/w) the membrane of
vesicle permeation increases thus drug entrapment decreases. At 30% ethanol the drug entrapment is high and at 10% ethanol it is less and above 30% of ethanol it again decreases.

Similarly the drug flux dependent on conc. Of ethanol, Up to 30% w/w of ethanol the drug flux increases when there is increase in ethanol concentration and decrease in flux conc. of drug as the ethanol conc. At higher concentration of ethanol there may be deteriorating effect on lipid bilayers which may lead to decrease in transdermal flux.

**Phospholipid**

Jain et al., reported that ethosomal size exhibited fever dependent on conc. Of phospholipids, there was significant increase in size of ethosomes with increase of phospholipids conc. to eight fold (from 0.5 to 4.0 %).

**Ethosomal Drug delivery Advantages:**

Ethosomal formulation has following advantages over dermal & transdermal delivery systems,

1. Drug delivery through skin by the dermal and transdermal route was increased by the Ethosomes which has increased the drug penetration through skin.
2. A large diversified group of drug belonging to various categories (peptides, Protein molecules etc) can be delivered by the Ethosomes.
3. They are the safe and approved for the cosmetic and for pharmaceutical formulation
4. As they are well documented in scientific literature about their toxicology they posses Low risk for the large scale formulation and drug development.
5. It is having high patient acceptability as they are means of easy administration of drug in semisolid form (gel or cream). On the other hand use of Iontophoresis and Phonophoresis having less patient acceptance than ethosomal formulation.
6. Due to proprietary technology these products attractiveness in market is high.
7. As there is no technical investment its manufacturing is simple.
8. It can be immediately commercialized as ethosomes system is non invasive and passive.
9. It has applications in various areas like Cosmetic, Pharmaceutical and Veterinary.
Characterizations of Ethosomes:

Visualization:

Ethosomes visualization can be done using electron transmission microscopy (TEM) and electron microscopy scanning (SEM)\textsuperscript{51}.

Zeta potential and Vesicle size:

Zeta potential and vesicle size can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS)\textsuperscript{52}.

Entrapment efficiency:

By the use of ultra centrifugation technique the efficiency of entrapment of the drug by the ethosomes can be measured\textsuperscript{53}.

Transition temperature:

Lipids transition temperature can be identified using differential scanning calorimeter\textsuperscript{54}.

Estimation of Surface tension:

In the aqueous solution the surface tension of the compound can be determined using Du Nouy ring tensiometer (ring method)\textsuperscript{55}.

Vesicle stability:

Assessing size and structure of the particles the stability can be determined. TEM is used to measure structure changes and DLS is used to measure mean size\textsuperscript{56}.

Penetration and permeation studies:

Ethosomes depth of penetration can be assessed by using CLSM (Confocal Laser Scanning Microscopy)\textsuperscript{57}.
Applications of Ethosomes:

Pilo sebaceous Targeting:

In the percutaneous delivery of the drug sebaceous glands and hair follicles are found to have potential significance. Further a good amount of attention is being given on exploitation of drug delivering shunt into systemic are follicles. For pilo-sebaceous targeting preparation and evaluation of Minoxidil formulation of Ethosomes was done by Maiden et al.\textsuperscript{58}

Transdermal delivery of hormones:

When hormones are administered orally, problems like low oral bioavailability, several side effects which are dose dependant and high first pass metabolism are observed and with each missed pill there is an increase in the risk of failure of treatment\textsuperscript{59}.

Anti-parkinsonism agent Delivery:

Ethosomal formulation with tri-hexyphenidyl hydrochloride (THP) is done which is a psychoactive drug and when compared to other formulations like liposomes drug delivery is greater. THP is used in treating Parkinson's disease and it is a M1 muscarinic receptor. These results proved better potential of permeation of skin by ethosomal THP formulation and that Parkinson's disease can be managed better by it\textsuperscript{60}.

Trans-cellular delivery:

Ethosomes as an anti- HIV therapy is found to be an attractive clinical alternative when compared to the other formulations in the market\textsuperscript{61}.

DNA delivery through Topical:

May pathogens from environment try to entering in to the skin/ Skin is known as a protective barrier which has the ability to express the gene and also which is active immunologically. Based on the above facts use of Ethosomes to deliver DNA molecules topically in order to express the genes in skin cells is said to be an important application. The possibility of usage of Ethosomes to deliver immunizing agents is due to the better ability of skin permeation of these dosage forms\textsuperscript{62}.
Anti-Arthritis drug Delivery:

Tropically when drugs like anti-arthritis are delivered seems to have much prominence due to its target specified delivery and problems which are found in conventional therapy are also overcome\(^6^3\).

Delivery of Antibiotics:

To enhance the therapeutic efficacy of antibiotics delivery of the drug by topical route is the better option. Several side effects and allergic reactions can be seen in conventional oral therapy. Low permeability into the deeper skin layers and sub dermal tissues are possessed by conventional external preparations.

1.6.2.3 TEST FOR EVALUATION:

1. Scanning electron microscopy study for filter membrane-vesicle interaction:

A filter membrane with a pore size of 50nm was applied with a vesicle suspension of (0.2ml) and kept into a diffusion unit. Upper side is exposed to air where as the (Phosphate saline buffer solution) PBS of pH 6.5 was in contact with the lower side. The removal of the filters was done after an hour and preparation was done for SEM studies by keeping it overnight in Karnovsky's fixative for fixation at 4 degree C and then by using ethanol graded solution (100% 95%,90%,70%,50%,30%v/v in water ) It was dehydrated. At last they were put a coating of gold and its examination was done in SEM\(^6^4\).

2. Skin permeation studies:

By the use of a pair of scissors, fur of animal under study is removed carefully (<2mm) and then by the use of a scalpel the abdominal skin and the underlying connective tissue was separated from each other. For the removal of fats and subcutaneous tissue the dermis of skin which was excised was kept on an aluminium foil. When the temperature of the cell was maintained at ±32 \(^0\)c where the cell permeation is 1.0 cm\(^2\) the receptor volume was 10ml. In the compartment of the receptor PBS (10ml of pH 6.5) was present.

In between donor and receptor the excised skin was moulded. 0.1ml Ethosomal formulation is applied on the skin surface and at 1,2,4,8,12,16,20 and 24 hrs interval 0.5ml of sample is taken from the diffusion cell through sampling port and assay is performed by using the HPLC.
3. Stability studies:

By storing particles at $4\pm 0^\circ C$ the determination of their stability was done. After duration of about 180 days the zeta potential, entrapment efficiency and particle size vesicles is estimated by appropriate method mentioned above.

4. SEM and TEM study for skin-Vesicle interaction:

Animals ultra skin sections were taken (Austria, Ultracut and Vienna) and placed on form vacated grids and then under transmission electron microscope its examination was done. For analysis by SEM the dehydrated sections of skin were placed on the stubs by the use of adhesive tapes and then gold palladium coat is coated by coater called fine coat ion sputter. Examination of section is done by electron microscope scanning.

5. Fluorescence microscopy study of skin-Vesicle interaction:

By following the protocol which is used for SEM and TEM fluorescence microscopy was carried out. For the micro cytotoxicity assay 5micrometer thin sections of paraffin using microtome are cut. At $37^\circ C$ and 5% CO$_2$, 2mmol/L L-glutamine, 100 U/ml penicillin, 100mg/ml streptomycin and 10% foetal calf serum containing solution which is called as Dulbecco's modified Eagle is used to examined T-lymphoid cell lines (MT-2 cells). 50% of absorbance decrease at 540nm is due to the cytotoxicity dose (CD50).

6. Drug uptake studies:

In 24 well plates (Corning Inc) MT-2 cells uptake the drug (1*106 cells/ml) is done by which an RPMI medium of 100 micro L was added. After incubating with 100 micro litre of solution of drug with cells, drug content is evaluated by HPLC assay and marketed after evaluated by using PBS (pH 7.4).

7. HPLC Assay:

MT-2 cell and Skin permeation can be determined by allowing the mobile phase flow at the rate of 1ml/min into the receptor compartment, where the mobile phase of HPLC contain 70:20:10 ratio of methanol: distilled water: acetonitrile respectively. The size of the C18 column was about 4.6*150mm
By the use of UV detector (SPDM10A VP diode) the column effluent was monitored at 271nm. The squared correlation coefficient was 0.9968 and 1.0%-2.3% was the standard curve variance coefficient.

8. Statistical analysis:

Studentized range test after the ANOVA is followed to find the data significance of all the data. Significance of P<0.05 is taken for the interpretation of prism results.

Experimental methods:

❖ Analytical methods of Carvedilol

1. Determination of $\lambda_{\text{max}}$ of Carvedilol in Potassium hydrogen phthalate (PHP) pH 7.4 buffer.

2. Calibration curve of Carvedilol in Potassium hydrogen phthalate pH 7.4 buffer.

❖ Pre-formulation studies:

1. Determination of melting point

2. Determination of partition coefficient

3. Permeability through rat skin

4. Optimization of various Carvedilol Ethosomal formulations

5. Polymer and skin compatibility

6. Determination of drug and polymer compatibility studies

❖ Formulation Design

1. Preparation of Ethosomes.

2. Preparation of Gel

❖ Evaluation of Ethosomal formulations

1. Physical appearance

2. Size and Shape analysis
3. Scanning electron microscopy
4. Entrapment efficiency
5. Drug content and uniformity
6. Skin irritation test
7. Dissolution studies.
8. Stability studies

METHODS:

Preparing standard calibration curve and to find λ max for Carvedilol.

Principle: Exhibits the Carvedilol absorption maxima at 242nm in potassium hydrogen phthalate

Procedure:

Preparation of Standard solution:

In methanol 10mg pure drug of Carvedilol was dissolved and this solution was diluted to give 10µg/ml concentrated solutions. Later it was scanned between 220nm and 300nm to determine wavelength. The wavelength of about 242nm was selected as maximum wavelength. To further analysis of drug solution same wavelength was used. It’s absorbance was measured at λ max 242nm.

Preparation of pH 7.4 phosphate buffer:

By dissolving 0.19gm of potassium dihydrogen phthalate, 2.38gm of disodium hydrogen phosphate and 8gm of sodium chloride in small quantity of distilled water and make 1000ml in a calibrated one later standard volumetric flask with distilled water.

Preparation of working Standard solution:

In a 10ml standard flask, 10mg Carvedilol in pure drug form was taken and it was dissolved in distilled water and the stock solution was made up to 10ml with a pH 7.4 phosphate buffer. Pipette a quantity of about 1ml of stock solution and transfer to 10ml
volumetric flask. Makeup to 10ml mark to give 100µg /ml Carvedilol in solution. From the same solution, different aliquots 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml quantities were taken in 10ml volumetric flasks containing buffer solution of phosphate pH7.4. Its volume was adjusted which gave resultant concentrations of 2, 4, 6, 8, 10 and 12µgm/ml. At 242 nm λ-max, the absorbencies were measured against blank. By taking absorbance on Y-axis and concentration on X-axis calibration curve was drawn which is taken as the standard.

**Pre-formulation studies:**

Before the design and development of Carvedilol Ethosomal gel formulation, pre-formulation studies were performed on various formulations namely stability, partition coefficient, melting point, diffusion rate constant on the drug Carvedilol.

**Partition coefficient:**

For the determination of substance lipophilic nature, water- oil partition coefficient is used. This in turn is further useful to predict whether substance is capable in crossing the biological membrane. The most popular method of finding partition coefficient is the shake flask method\(^2,^3\).

**Procedure:**

In to 5 ml of n-Octanol, a small quantity of Carvedilol was added to get a saturated solution. It was passed through filter paper to obtain a clear solution. A 3 ml portion was mixed with 2ml of fresh n- Octanol. Taking 15ml of water at 37\(^o\)c 5ml of prepared solution of Carvedilol in n-Octanol was mixed well for 24hrs on cryostat temperature shaker bath.

Using UV Spectrophotometric method the drug contents in the separated phases namely water and n-Octanol phases is estimated by making suitable dilutions and by employing the formula its partition coefficient (Kp) is found.

\[
Kp = \frac{C_{org}}{C_{aq}}
\]

Where

\(C_{org}\) is drug content in organic phase

\(C_{aq}\) is drug content in aqueous phase
Melting point:

Melting point (MP) of Carvedilol pure drug was determined by the closed capillary tube method. The temperature at which the drug melted was noted. The melting points are tabulated in table.

COMPATIBILITY STUDIES:

For the investigation and prediction of any physicochemical interactions between varied components of a formulation, IR spectroscopy can be used and thus can be useful in selecting chemically compatible excipients which are suitable.

The goal of this investigation was to detect carrier-drug interactions if any. IR spectroscopy for the following compounds was recorded.

- Carvedilol
- Propylene glycol
- Soya lecithin
- Carbopol
- Ethanol
- Cholesterol
- Ethosomal gel
- Triethanolamine

**Carvedilol Ethosomal gel:**

In a mortar one part of sample and three parts of potassium bromide were taken and triturating was done. Using hydraulic press a small quantity of sample which was triturated was put into machine. Then it was compressed at a pressure of 10kg/cm². In Bruker IR spectrophotometer the pellet was scanned from 4000/cm to 400/cm then its comparison was done with the original spectrum.

To find if there is any shifting in functional peaks and no involvement of functional group, IR spectra was checked thoroughly and even compared with the original one. From the
observations it is clearly found that there are no interactions between the drugs, mixtures and selected carriers. Hence compatibility was seen between the selected Carvedilol and carrier without mutual interactions.

**Polymer skin compatibility:**

The skin irritation was done to investigate the polymer compatibility with the skin. The following procedure was adapted to healthy albino rats of average weight between 230-250mg and the skin irritation test was conducted. 0.5% of aqueous solution was used as a standard irritant.

Polymeric solution were spread over $1 \text{ cm}^2$ on each rat on its dorsal surface on left side and on right dorsal side. 0.5% formalin was applied. After 24hr, alcohol swabs were used to remove the polymers followed by identification for the presence of edema/erythema and data was tabulated in a table.

**FTIR studies:**

Infrared spectroscopy is more applicable in the qualitative identification of substances whether pure or in mixture. It is an excellent tool to establish the structure of new compounds. The IR spectrum is useful to get detailed information about the structure of molecular compounds. By comparing the IR spectra of the test substances and the pure compound one can establish the authenticity of substances.

The infrared data aids in conforming the identity of the drug and detects the information of the drug with polymers. The infrared spectra of Carvedilol and polymers, alone and in physical mixture were taken. Investigation was done to determine any interaction between polymer and Carvedilol. Spectral data are show in spectra and their results are tabulated in table.
Formulation Design:

Preparation of Sonicated Carvedilol Ethosomes (by Cold method):

Making slight modification to the Touitou et al method Carvedilol Ethosomes were prepared\textsuperscript{13}.

The Carvedilol Ethosomal system comprised of 20-50% of ethanol, 10% of propylene glycol, 2-5% of phospholipids, 0.005g of cholesterol and an aqueous part of 100% w/w. At room temperature, 0.025g of Carvedilol was added to ethanol in a covered vessel along with propylene glycol and dissolved by stirring it vigorously. At 30\textdegree C mixture was heated using separate vessel and then drop wise it was added to the mixture in the centre of the vessel by stirring it at 700rpm for 5min in a vessel which was covered.

Then by using extrusion\textsuperscript{30} method or sonication\textsuperscript{30} method the particle size of Ethosomal formulation was reduced to the desirable extent. At last the Ethosomal formulation was kept under refrigeration. The following process was used to prepare Ethosomes spontaneously.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure28.png}
\caption{Sonicated Ethosomes preparation by cold method.}
\end{figure}
**Preparation of Unsonicated Carvedilol Ethosomes:**

By the minor modification to Touitou et.al. Method, unsonicated Carvedilol Ethosomes were prepared.

The Ethosomes consisted of 2 – 5% phospholipids, 20-40% ethanol, 0.005 % of cholesterol, 0.025gm of Carvedilol and distilled water quantity sufficient 100% w/w at room temperature. The mixture was heated to 30\(^\circ\)C and continuous stirring with 700RPM for 5 minutes is added drop wise in a closed i.e. minutes vessel for 5 min.

**Preparation of Carvedilol Liposomes:**

The cast film method was adopted for the formulation of Carvedilol liposomes. The formula employed for the preparation consisted of phospholipids 2%, and cholesterol 0.005gm and drug 0.025gm.

All these ingredients were dissolved with the aid of a small quantity of chloroform in a round bottomed flask (RBF) like a thin film. It is formed inside the flask when the chloroform is removed by using vacuum. Using dilute HCl the film obtained was hydrated which was made up to 20gm w/w with water.
## ETHOSOMAL FORMULATIONS:

<table>
<thead>
<tr>
<th>S.NO</th>
<th>INGREDIENTS</th>
<th>SONICATED ETHOSOMES</th>
<th>UNSONICATED ETHOSOMES</th>
<th>LIPOSOMES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EF1</td>
<td>EF2</td>
<td>EF3</td>
</tr>
<tr>
<td>01</td>
<td>Drug (g)</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>02</td>
<td>Lecithin (Soya lecithin %)</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>03</td>
<td>Ethanol (%)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>04</td>
<td>Propylene glycol (%)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>05</td>
<td>Cholesterol (g)</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>06</td>
<td>Water</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
</tr>
</tbody>
</table>
### Table-9: Composition of different Sonicated Carvedilol Ethosomes and Liposomes

<table>
<thead>
<tr>
<th>Ethosomal formulation</th>
<th>Lecithin (Soya lecithin %)</th>
<th>Ethanol (%)</th>
<th>Propylene glycol (%)</th>
<th>Drug (g)</th>
<th>Cholesterol(g)</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF1</td>
<td>2</td>
<td>20</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF2</td>
<td>3</td>
<td>20</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF3</td>
<td>4</td>
<td>20</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF7</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
</tbody>
</table>

### Table-10: Composition of different Unsonicated Ethosomes and liposomes

<table>
<thead>
<tr>
<th>Ethosomal formulation</th>
<th>Lecithin (Soya lecithin %)</th>
<th>Ethanol (%)</th>
<th>Propylene glycol (%)</th>
<th>Drug (g)</th>
<th>Cholesterol(g)</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF4</td>
<td>2</td>
<td>20</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF5</td>
<td>3</td>
<td>30</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF6</td>
<td>4</td>
<td>40</td>
<td>10</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
<tr>
<td>EF7</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>0.025</td>
<td>0.005</td>
<td>q.s</td>
</tr>
</tbody>
</table>
Preparation of Ethosomal gel of Carvedilol:

In the Carbopol gel of various compositions (1%, 1.5%, 2% w/w) the best achieved Ethosomal suspension, of formula was selected and was incorporated specific amounts of Carbopol 934 powder which were added slowly to ultra pure water and kept for 20mins at a temperature of 100°C. Then later on, drop wise Triethanolamine was added into it.

Then incorporation of an accurate quantity of formula which contains Carvedilol (1.5% w/w) was done in the gel base and then water of sufficient quantity was added with continuous stirring to the other ingredients of the formulation until a formulation of homogenous nature was obtained which were christened as (G-1, G-2, G-3). By the use of 1.5%w/w Carbopol, a gel constituting free Carvedilol was prepared with the aid of similar method.

<table>
<thead>
<tr>
<th>Gel formulation</th>
<th>Carvedilol Ethosomal suspension(ml)</th>
<th>Carbopol (%)</th>
<th>Triethanolamine (ml)</th>
<th>Phosphate buffer (pH 7.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*G-4</td>
<td>0.025g</td>
<td>1.5</td>
<td>0.5</td>
<td>q.s</td>
</tr>
<tr>
<td>G-3</td>
<td>20</td>
<td>2</td>
<td>0.5</td>
<td>q.s</td>
</tr>
<tr>
<td>G-2</td>
<td>20</td>
<td>1.5</td>
<td>0.5</td>
<td>q.s</td>
</tr>
<tr>
<td>G-1</td>
<td>20</td>
<td>1</td>
<td>0.5</td>
<td>q.s</td>
</tr>
</tbody>
</table>

*G-4 Drug free gel.
CHARACTERIZATION OF ETHOSOMES and LIPOSOMES:

SIZE AND SHAPE ANALYSIS:

For the determination of Ethosomes, average size microscopic analysis was done. A sample of Ethosomes was taken and it was diluted by using distilled water. For the examination at 45×15 X microscope on a glass slide diluted drop is taken which is then covered with a cover slip. By using calibrated eyepiece micrometer with stage micrometer the diameter of 150 vesicles was determined. The formula used for calculating average diameter was

\[
\text{Average diameter} = \frac{nd}{n}
\]

Where

- \( n \) = number of vesicles
- \( d \) = diameter of vesicles

The vesicle size was decreased by Sonication method because size of these vesicles were unable to be analyzed using the microscopic method at a magnification of 15×45 X. So the Sonicated vesicles were analyzed under a special microscope which has a connection with software and under 400 and 800 magnification its microphotographs were taken. Further few microphotographs were selected and analyzed for size by using a particular software “particle size analysis” which was developed by BIOVIS. This special software is working on microphotograph images with standard dimension\(^{14,15}\).

SCANNING ELECTRON MICROSCOPY:

Determination of morphological characters like its texture of smoothness, round structure and aggregate gel formation of Carvedilol Ethosomes was done using scanning electron microscopy\(^{16,17,18}\).
ENTRAPMENT EFFICIENCY:

A 10ml aliquot of Carvedilol Ethosomal suspension was ultra centrifuged to determine entrapment efficiency. For every 2 minutes gap all the samples were vortexes for 5 minutes each cycle at least of 2 cycles were done.

Then from all the samples which have been vortexed a quantity of 1.5ml is taken and Ethosomal formulations which are fresh and untreated were put into different tubes of centrifuge. The centrifugation of the samples was done at a rate of 20,000 rpm for duration of 3 hours. Separation of the supernatant layer was done and diluted with a suitable quantity of water and then absorbencies’ for the respective concentration of the drug were found at 242nm in both un-vortexed and vortexed samples.

The efficiency of entrapment was calculated accordingly:

\[
\text{Entrapment efficiency} = \frac{T-C}{T} \times 100
\]

Where T denotes detected total drug from the vortexed supernatant sample. C denotes unvortexed detected drug supernatant sample and amount of drug entrapped.
Figure 30: Magnetic Stirrer with Franz Diffusion Cell
### Table-12: Ethosomal Formulation Characterization Methods

<table>
<thead>
<tr>
<th><strong>METHODS</strong></th>
<th><strong>PARAMETERS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Staining with Eosin-Hematoxylin</td>
<td></td>
</tr>
<tr>
<td>Electron Transmission microscopy</td>
<td>Study of Skin-Vesicle interaction</td>
</tr>
<tr>
<td>Fluorescence microscopy</td>
<td></td>
</tr>
<tr>
<td>Confocal laser scanning microscopy</td>
<td></td>
</tr>
<tr>
<td>Dynamic light scattering method</td>
<td>Size distribution and Vesicle size</td>
</tr>
<tr>
<td>Fluorescence spectrophotometry</td>
<td>Efficiency of Entrapment</td>
</tr>
<tr>
<td>Mini column centrifugation method</td>
<td></td>
</tr>
<tr>
<td>Transmission electron microscopy</td>
<td></td>
</tr>
<tr>
<td>Scanning electron microscopy</td>
<td>Morphology (Shape) of Vesicle</td>
</tr>
</tbody>
</table>

### CHARACTERIZATION OF GEL:

**Surface morphology:**

The scanning electron microscope was employed by utilizing the gold sputter technique to detect the morphology of the surface of Ethosomes was done. The system was dried under vacuum and coated with gold palladium and then microscopically observed.

**Organoleptic Characteristics:**

The psycho rheological properties of the formulations were tested like the colour, odour, phase separation, feel upon application (greasiness, grittiness) and texture.
**Wash ability:**

On to the skin a small amount of gel was applied and then it was washed with water and checked whether the gel was able to be totally washed or not.

**Spread ability:**

The determination of Spread ability was done by glass slide apparatus and modified wooden block. A specified amount of the gel was put over the movable pan with a slide of glass affixed to it. It was then kept on the fixed slide made of glass so that the gel was sandwiched in between the two slides of glass for duration of 5 min. The continuous removal of weight was also done. The determination of Spread ability was done by using the following formula:

\[ S = \frac{M}{T} \]

where,

- **T** denotes Time in seconds,
- **M** denotes Mass in grams,
- **S** denotes Spread ability in g/s.

**pH:**

A solution of 1 gm of gel was prepared which was dissolved in distilled water of 30 ml with a pH 7. By bringing contact of the probe of the pH meter with the samples in a digital pH meter the pH of the Ethosomal gel was determined.

**Content uniformity and Drug content:**

Dissolve 1 gm gel in 100 ml of pH of 7.4 phosphate buffer for duration of about 48 hrs and by using magnetic stirrer constantly stir the mixture. Then filtration of the solution was done and UV spectrophotometer was used to observe it at a maximum wavelength of 242 nm. Triplicate measurements were made.
Skin irritation test:

Male Wister rat was taken and the hair over its abdominal skin was clipped. 24hrs before applying the formulated product to the skin (hairless), 0.5g of the same is introduced evenly spreading it over an area of 4cm². After 24, 48 and 72 hrs of the application of formulation it was observed for inflammation based on the degree of reduces.

Based on erythematic degree the mean erythemal scores were recorded.

4=Extreme red (indicate the severe Erythema)

3= Dark pink to light pink colour (indicate Erythema moderate to severe)

2= Dark pink (indicate Erythema moderate condition)

1= Pale or light pink (slight Erythema)

0=No change in colour ( no Erythema : Legend )

Figure31: Skin Irritation test
SKIN PERMEATION STUDIES:

COLLECTION OF RAT SKIN:

To study the permeability via skin of Carvedilol Ethosomal gel the skin above of the stomach of a male Wistar rat was freshly shaved and made hairless, weighing 250g and of 8 weeks was kept individually by providing ad libitum food and water in animal house. Firstly by using the technique of decapitation the rat was sacrificed and then the hairs of the abdominal region with the aid of scissors were carefully cut into short.

Then the skin was excised surgically and cleaned of any vasculature or fat present and should be kept at a temperature of $-4^\circ C$ for 24hrs prior to the experiment. On a modified Franz diffusion cell of area $0.785\text{cm}^2$ the samples of skin were mounted in such a way that compartment of donor is in contact to $S$.corneum, the surface of the dermal skin which is exposed to the receiver phase.

![Figure 32: Bio-pack Machine](image)

DRUG RELEASE STUDY FROM RAT SKIN:

Specifically improvised laboratory model of Franz diffusion cell. According to the literature studies of Carvedilol Ethosomes, formulation of Skin permeation is carried out. The receptor cell volume was 20ml and the area of permeation of the cell which was effective was about $2.4\text{cm}^2$ and $37 \pm 0.5^\circ C$ temperature is adjusted.
Using the Magnetic stirrer at 100RPM the 20ml of solution whose pH is 7.4 is continuously stirred. Rat Skin is placed in between donor and receiver. On the rat skin was placed, 1gm of formulation of Ethosomes is applied on diffusion cell maintained with constant stirring and it is also applied on epidermis skin of the rat. Then samples of 1ml quantity were taken out of diffusion cell receptor compared at predetermined time intervals. They were analysed by spectrophotometer method at 242nm after a suitable dilution. Immediate replenishment of the receptor phase with a volume of equal quantity of fresh pH 7.4 buffer was done. For the permeation studies of the skin, triplicate experiments were conducted.

![Figure 33: Albino Wistar rats prepared in vitro release studies](image)

**DISSOLUTION STUDIES:**

Male Wister rats weighing 230-250grms were procured from a vendor approved by ethical committee, then kept for stabilization under prescribed conditions of laboratory such as 12hr day light, relative humidity of 55±5% and temperature of 25±1 °c were maintained
and polypropylene cages were used to house animals containing 4 in each cage. Following the IAEC guidelines, the animals were freely allowed for the water and pellet diet (Lipton feed, Mumbai) to ad libitum during the in-vivo studies.

Wister rats with no abnormalities on skin were selected for the Ethosomes Pharmacokinetic evaluation for bioavailability. The animals weighing between 230-250 grams were only used for the study; their skins were shaved on dorsal side for an area of 10 cm². 24hrs prior to the formulation application and were under observation for untoward effects for 24hrs. This was carried out while the rats were in fasting condition.

Three groups of 6 animals each were divided which were given following treatment. For the 1st group oral 5mg/kg Carvedilol was administered, 2nd group was treated with F1 Ethosomes and 3rd group received F2 Ethosomes. After regular intervals blood was collected i.e.; at 1st, 2nd, 5th, 8th, 12th and 24th hour and were subjected to centrifugation to collect plasma for the Carvedilol drug estimation which was done later on. Till then plasma was stored at -70°C. Based on RP-HPLC the Carvedilol concentration was estimated after slight modifications.

By adding 1N NaOH (50 µL) plasma samples converted to alkaline and by diethyl ether (5 ml) extraction was done. In 250 µL of 0.2% phosphoric acid analysts were back-extracted. In the RP-HPLC system analyses of 50 µL were injected. Liquid chromatography LC-10A model assembly (Shimadzu, Japan), along with fluorometry detector of RF-10A and rhodyne 7125 injector where the detector was set to 278-320nm emission filter were used. The C-18 base-deactivated column (5 µm, 150 × 20 mm). The mobile phase that consists of (1). Proportionate mixture of Iso-propanol, acetonitrile, and methanol in ratio (1:2:7) (2). Potassium phosphate buffer 20 mm pH 2.5 was used. The 31% of mobile phase is increased to 47% within 8minutes from the start, for 3minutes its percentage is held in this manner. Later on its condition is retained in next 12 minutes.

Stoelting, Wood Dale, II with a digital display and tail cuff mode is the B.P. measuring apparatus used to check the blood pressure in rats to find out the effect of transdermal Ethosomal gel in comparison with hypertensive rats. The rats were tamed and used for the study in such a way that they stay calm in the rat holder during the B.P. measurement. Prior to the hypertension induction by MPA 20mg/kg/week (subcutaneously) the normal B.P. of each animal is recorded.

After two weeks of dosing of MPA animals are grouped into 4 groups containing 6 in each group, the animals with 150mm Hg is the criteria for the selection of animals. 1st group is taken as a control which is treated with only MPA, 2nd group is treated as standard which is
treated with oral dose of Carvedilol. 3rd group is treated with F2 gel of Ethosomes and 4th group is treated with F6 gel of Ethosomes and at different time durations B.P is measured i.e. at 1,2,4,6,10, and 12 hours.

**Skin irritation test:**

One day before the commencement of study the hair on the dorsal side of the rats is removed by clipping and grouped into 4 groups consisting of 6 animals each. Control is the 1st group that receives toxicant only, trans-dermal ethosomal gel F2 is administered to 2nd group, 3rd group is treated with trans-dermal Ethosomal gel F1 and 4th group is standard group that is treated with 0.8% v/v formalin aqueous solution. This treatment is done for 7 days and grading is done based on the visual scoring made by the investigator.

**EVALUATION TESTS:**

1. **Scanning electron microscopic studies for vesicle- Filter membrane-interaction study:**

   A filter membrane with a pore size of 50nm was applied with a vesicle suspension of (0.2ml) and then introduced into the diffusion cell. The upper side was the one which air exposed. Whereas the lower side container pH 6.5 (Saline buffer solution of Phosphate).

   Filters were removed after an hour and preparation was done for SEM studies by keeping it overnight in Karnovsky's fixative for fixation at 4 degree C and then by using ethanol graded sol. (100% 95%, 90%, 70%, 50%, 30% v/v in water) it was dehydrated. Finally filters were put a coating of gold and its examination was done in SEM (Leica, Germany, Bensheim).

2. **Skin permeation studies:**

   By the use of a pair of scissors Wistar rats hairs were cut short carefully (<2mm) and then by the use of a scalpel the abdominal skin and the underlying connective tissue was separated from each other. On aluminium foil the excised skin is kept and the removal of subcutaneous and adipose tissue was done, which acts like diffusion cell with an area of diffusion 1.0cm$^2$ and it has 10 ml effective permeation volume at 32±1$^0$ C. Temperature and PBS (10ml of pH 6.5) buffer. After placing the skin between compartment receptor and compartment donor, Ethosomal formulation of 1.0ml was applied on skin epidermis. About
Samples of 0.5ml were withdrawn from the diffusion cell at various time intervals of about 1, 2, 4, 8, 12, 16, 20, 24 hrs and the samples were assayed by HPLC method.

3. Stability study:

By storing the particles at 4± °C the determination of their stability was done. After duration of about 180 days the particle size, zeta potential and entrapment efficiency are estimated by the earlier described methods.

4. Vesicle-skin interaction study by SEM and TEM:

Very fine skin sections of rats (Vienna, Austria, Ultracut) were placed on firm var-coated grids and then under transmission electron microscope its examination was done. For analysis by SEM the dehydrated section of skin were placed on the stubs by the use of adhesive tapes and then section was coated by fine coat ion sputter coater the sections were coated with gold palladium then finally by using scanning electron microscope the examination of the sections was done.

5. Skin interaction study by Fluorescence microscopy:

By following the protocol which is used for SEM and TEM fluorescence microscopy was carried out. By using a microtome the paraffin blocks were cut to be made into thick

Figure 34: Stability chamber
sections of 5micro metre and then under a fluorescence micro cytotoxicity assays it was examined. In the Dulbecco’s Modified Eagle medium, MT-2 cells are placed where the medium consists of 2mmol/L L-glutamine, (10%) foetal calf serum, 100mg/ml Streptomycin, and 100 U/ml Penicillin. This medium is maintained at 37\(^0\)C with 5% co2. Cytotoxicity which is expressed in the form of cyto-toxic dose 50 (CD 50) that has resulted in 50% decrease in absorbance at 540nm.

6. **Drug uptake studies:**

   In 24 well plates (Corning Inc) MT-2 (1*10^6 cells/ml) cells uptake of drug done by RPMI medium of 100 µL was added. The drug content was assayed by HPLC method. With the marketed formulation or PBS (pH 7.4) or in Ethosomal formulation the drug solution of 100 µL was used to incubate cells.

7. **HPLC Assay:**

   By using HPLC method with mobile phase of Methanol: distilled water: Acetonitrile in a ratio of (70:20:10) delivered with a 1ml/min flow rate. Drug amount which has permeated in the compartment of the receptor while MT-2 cell and skin permeability in-vitro studies can be evaluated. The elution of a twenty micro litre injection in C-18 column was done at room temperature. The size of the C18 column was about 4.6*150mm. By the use of SPDM10A VP diode array UV detector the column effluent was monitored at 271nm. The squared correlation coefficient was 0.9968. The coefficient of variation was found to be 1.0% to 2.3%.

8. **Statistical analysis:**

   For the determination of data significance Studentized range test and ANOVA was employed for the statistical analysis. To interpret the results prism, a significant of P<0.05 was frozen as the confidence limit.

**In-vitro release kinetics:**

The dissolution pattern obtained for all the formulations were graphically plotted for the following parameters namely.

- Peppa’s model / equation of Korsmeyer – % drug released Log cumulative v/s log time.
- Higuchi’s model – Square root of Time v/s Cumulative % drug released.
• First – order kinetic model – time v/s Drug remain log cumulative %.
• Zero - order kinetic model – Time v/s Cumulative % drug released.

**Zero order kinetics:**

Zero request discharge will be anticipated by the accompanying mathematical statement:

\[ A_t = A_0 - K_0t \]

Where,

\[ A_t = \text{Drug discharge at time’}t’ \]
\[ A_0 = \text{Initial medication fixation.} \]

**First order kinetics:**

Initially - request discharge could be anticipated by the accompanying comparison:

\[ \log C = \log C_0 - K_1t / 2.303 \]

Where,

\[ C = \text{Medication Amount stayed at’}t’ \]
\[ C_0 = \text{Medication Initial measure.} \]
\[ t = \text{time} \]

A straight line is plotted when log aggregate of percent medication remaining is plotted against time. When Slant worth duplicated with 2.303, steady "K1" is obtained.

**Higuchi’s model:**

Higuchi’s equation for classical diffusion explains the matrix devices drug release pattern by diffusion:

\[ Q = \frac{D\varepsilon}{\tau (2A - \varepsilon C_s) C_0^2 t} 1/2 \]

Initially - request discharge could be anticipated by the accompanying comparison: the various notations are
Q = Amount of medication discharge at ‘t’
t = number of hours elapsed when measure of medication is discharged
Cs = Medication Solubility in network
A = In unit network volume total medication content
D = Medication coefficient of Diffusion in network.

At the point when log aggregate of percent medication remaining is plotted versus time gives a straight line. The steady "K1" can be obtained by duplicating 2.303 with the slant worth.

Medication discharge from the network gadgets by dispersion has been depicted by emulating Higuchi's traditional dissemination mathematical statement:

Above comparison can be rearranged as though we expect that 'D', "Cs" and "An" are steady. At that point mathematical statement gets followed.

At the point when the information is demonstrated as per mathematical statement i.e. aggregate medication discharge against foundation time square gives straight line of a, slant is equivalent to "K" (Higuchi's 1963) defines dissemination component medication discharge.

**Peppa’s model/ Korsmeyer equation:**

For the elucidation of Liposomal formulation drug release pattern and exponential comparison of drug discharge (Peppa's law comparison /Korsmeyer mathematical statement) is employed, which is used for the polymeric medication release pattern.

Where

\[
\frac{M_t}{M_\alpha} = \text{the portion of medication discharged at ‘t’}.
\]

\[
n = \text{Type of diffusion identified with the instrument of discharge}
\]

\[
K = \text{Polymer /drug framework geometrical and structural Constant.}
\]

This study has created that expanded admission of sodium salt prompts a rise of systolic pulse in the test animals.

**IN-VIVO PERMEATION STUDIES:**

Animal house facility of Smt. Sarojini Ramulamma College of pharmacy provided after clearance by Institutional animal ethics committee (IAEC) 8 weeks old, 36 Wistar Male rats, of 230- 250gm weight. The IAEC proposal number issued was
A copy of the same has been enclosed. Conditions of standard laboratory were maintained for housing of test animals in the Pharmacology laboratory i.e; Temperature of $25 \pm 2^\circ c$ with light/ dark cycle of 12 hr each was maintained. Pellet diet supplied by Lipton India was given followed by ad libitum water. Picric acid solution was used for the marking of animals for their identification.

The experimental method were performed according to the norms of the CPCSEA and IAEC

**CONDITIONING AND TRAINING OF ANIMALS:**

The BP measurements studies were conducted on the rats which were kept in restrainers rat holders which were designed as follows entry/exit is from one side only with sufficient ventilation on other sides in order to acclimatize the rats to this type of environment the test animals was kept in the cage headlong in order that its gets accommodated comfortably and the cage locked so that tail was left outside on the open side.

All the animals were trained a number of times until they got accustomed to reside the cages in a non aggressive manner.

**SYSTOLIC MEASUREMENT OF RATS:**

A. **Methyl prednisolone (MPA)acetate induced method:**

Non invasive blood pressure apparatus manufactured by Bio-pack system inc. Santababara, USA was utilized for measurement of BP of all the rats. To the restrainers holder in which the tail of the rat was protruding out, tail cuff method was adopted to measure the systolic pressure. Average of three consecutive readings was noted.

**INDUCTION OF HYPERTENSION IN NORMAL RATS, BY MPA AND SYSTOLIC BP MEASUREMENT IN RATS:**

Grouping of animals is done into 6 groups containing 6 animals in each, where group I is control and rest of the groups are induced with subcutaneous MPA
20mg/Kg for hypertension. Group II was designated as MPA control Group III was administered EF2 20mg/Kg. Group IV is treated with EF4 20mg/Kg. Group V is treated with EF7 20mg/Kg. Group VI is treated FD (marketed drug) 20mg/Kg.

The systolic BPs were measured for all the groups initially, after 1hr, after 2 hr, after 4hr, after 6hr, after 10hr and after 12hr. these BP measurements have been compiled in a comprehensive comparative table which is presented below.

B. Sodium induced method:

Non invasive blood pressure apparatus manufactured by Bio-pack system inc. Santabarbara, USA was utilized for measurement of BP of all the rats. To the restrainer holder in which the tail of the rat was protruding out, for the B.P estimation of rat’s tail-cuff method was employed. Average of three consecutive readings was noted.

INDUCTION OF HYPERTENSION IN NORMAL RATS, BY MPA AND SYSTOLIC BP MEASUREMENT IN RATS:

The study design consists of 6 groups consisting of 6 animals in each group where treatment of these groups is as follows. Control group (Group I) they are normal and in the other 5 group’s hypertension were induced by subcutaneous injection of 20mg/Kg body weight. Group II was designated as MPA control, Group III is treated with EF2 20mg/Kg. Group IV is treated with EF4 20mg/Kg. Group V is treated with EF7 20mg/Kg. Group VI is treated with FD (marketed drug) 20mg/Kg body weight.

The systolic BPs were measured for all the groups initially, after 1hr, 2 hr, 4hr, 6hr, 10hr and 12hr. These BP measurements have been compiled in a comprehensive comparative table which is presented below.