FORMULATION AND EVALUATION OF ETHOSOMES OF CLOBETASOL PROPIONATE

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ABSTRACT

Ethosomes are vesicular drug delivery systems. “Ethosomes are ethanolic liposomes”. Ethosomes are lipid vesicular structure containing phospholipids, alcohol in relatively high concentration, propylene glycol and water. Ethosomes can entrap drug molecule of various physicochemical characters such as hydrophilic, lipophilic or amphiphilic in nature. The size range of ethosomes may variate from tens of nanometers to microns. The advantage of ethosomes over other topical delivery includes enhanced permeation of drug molecules to the skin and through the skin to the systemic circulation. Better patient compliance. Better stability and solubility of many drugs as compared to conventional vesicles. Relatively smaller size as compared to conventional vesicles. Composition and components of ethosomes are safe, they have various applications in pharmaceutical, veterinary and cosmetic field. Clobetasol propionate is a synthetic corticosteroid, for topical dermatologic use. It is derived from prednisolone with low mineralocorticoid activity and high glucocorticoid activity. Corticosteroids are therapeutic agents generally used in the treatment of skin disorders such as psoriasis and eczema. As research shows that stratum corneum is the main barrier for topical absorption of drug. Topical application of drug at pathological sites offers possible advantages of delivering the drug directly to the site of action. The present study aim to formulate and evaluate ethosomes of clobetasol propionate. Ethosomes of clobetasol propionate was successfully prepared by cold method and characterized by pH, size, zeta potential, drug content, drug entrapment, in-vitro drug release and formulation F9 was found to be best from all the formulations prepared.

KEYWORDS: Ethosomes, liposomes, amphiphilic, glucocorticoid, stratum corneum, topical, in-vitro.
INTRODUCTION

Topical drug delivery is an attractive route for local and systemic delivery of drugs. The topical drug delivery system is applied to the body through ophthalmic, vaginal, rectal and skin as topical routes. Skin is one of the most widely accessible organs on human body for topical drug delivery system. The clinical evidence shows that the topical gels are safe and effective treatment for the management of local dermatologic diseases. Drugs applied to the skin for their local action includes antiseptics, analgesics, antimicrobial agents, skin emollients and protectants.

Stratum corneum is the main barrier for the percutaneous absorption of topically applied drug onto the skin. In ethosomes, ethanol act as a penetration enhancer through the skin. Ethanol enables the drug to reach into intercellular lipids and induces the fluidity of cell membrane lipids and reduces the density of lipid bilayer of cell membrane which results increased skin permeability. So ethosomes penetrates very easily to the deeper layers of skin, where it combined with skin lipid contents and releases the API into inner layers of skin.

Merits of Topical Drug Delivery

1) It can penetrate deeper into skin and hence give better absorption.
2) They can avoid gastrointestinal drug absorption difficulties and first pass metabolism of active pharmaceutical ingredient.
3) They can alternative for oral administration of medication when the oral route is unsuitable, as due to vomiting and diarrhea.
4) They are non invasive, avoiding the inconvenience of parenteral therapy.
5) High patient compliance.
6) It shows lower fluctuations in plasma drug levels.
7) Improve the patient compliance.
8) Provide suitability for self-medication.
9) Ability to easily terminate medications, when it is needed.
10) Ability to deliver the drug which is more selective for a specific site.

Demerits of Topical Drug Delivery Systems

1. Skin irritation may occur due to contact dermatitis by the drug and excipients.
2. Poor permeability of some drugs through the skin.
3. Possibility of allergenic reactions.
4. Used only for those drugs which require very small plasma concentration.
5. Larger particle size of the drugs are not easy to absorb through the skin.

ANATOMY OF SKIN
Skin is the largest organ in the body. The skin of an average adult human covers a surface area of nearly 2 m² (or 3000 in²) the weighs 4.5-5kg (10-11 lb), about 16% of total body weight and receives about one-third part of the blood circulating throughout the body. It is elastic, rugged and under normal conditions, self regenerating with a thickness of only a few millimeters (2.97±0.28 mm). Structurally skin consists of two main parts. The superficial, thinner portion, which is made up of epithelial tissue, is the epidermis and the deeper, thicker connective tissue portion is dermis, also called as hypodermis, this layer consist of areolar and adipose tissues.

Microscopically the skin is a multilayered organ made up of three tissue layers:
- The epidermis
- The dermis
- The subcutaneous fat tissue.

![Layers of Skin](image)

**Fig 1: Layers of Skin.**

**Epidermis**
The epidermis is the most outer layer of the skin. There are no blood vessels and no nerve endings present in the epidermis. The epidermis is divided into two layers: the stratum corneum and the stratum germinativum. The stratum corneum is the primary barrier to percutaneous absorption. The stratum corneum consists of multiple layers of compacted, flattened, dehydrated and keratinized cells in stratified layers. These horny cells have lost
their nuclei and are physiologically rather inactive. They are continuously replaced by the stratum germinativum which is regenerative layer of epidermis. Normally, stratum corneum cells have water content of approximately 20% and in stratum germinativum the cells have water content of 70%.

The stratum corneum is made by three layers, the stratum spinosum (pricky layer), the stratum granulosum (granular layer), the stratum lucidum (clear layer). Removal of these three layers results in water loss and an enhanced skin permeability.

**Fig 2: Layers of Epidermis.**

**Stratum Corneum**
Consist of an average about 25 to 30 layers of the flattened dead keratinocytes. The horny pads are at least 40 times thicker than membranous horny layer.

**Stratum Germinativum**
Is the high mitotic index for cells layer and constantly renew the epidermis and these proliferations in a healthy skin balance the loss of the dead horny cells from the skin surface.

**Stratum Granulosum**
They are manufacturing the staining particles of keratinohylline granules.

**Stratum Spinosum**
The layers of these cells are produced by the morphological and histochemical alteration of the cells of basal layers.
Stratum Lucidum
Present in only thick skin of areas such as the fingertrips, sole of the foot and palm of the hand.

Malpighion Layer
The basal cell includes melanocytes which produce melanin granules to distribute the keratinocytes required for the pigmentation of protective measure against radiation.

The epidermis is consists of keratinized stratified squamous epithelium, that protects the underlying tissues, consist of four type of cells.

- Keratinocytes cells
- Melanocytes cells
- Langerhans cells
- Merkel cells

Fig 4: Types of cells present in epidermis.

Dermis
The deeper, thicker connective tissue portion is dermis, also called as hypodermis, this layer consist of areolar and adipose tissues. Microscopic examination shows that dermis is made up of a network of robust collagen fibres of fairly uniform thickness (0.2-.03 cm) with regularly spaced cross-striations. This network is a gel containing polypeptide macromolecules which is responsible for the flexible properties of the skin. It is divided into two distinct zones: a superficial finely structured thin papillary layer adjacent to the epidermis containing blood vessels, lymphatics and nerve endings; and a deeper coarse reticular layer (the main structural layer of skin).
Subcutaneous Tissue
This is a sheet of fat containing areolar tissue (superficial fascia), attaching the dermis to the underlying structures. It acts as a heat insulator and a shock absorber. The subcutaneous layer act as a strong depot fat it contains large blood vessels that supply to the skin.

MATERIAL AND METHODS
Clobetasol propionate was received as a gift sample from Rextin Pharmaceutical PVT. LTD. Dialysis membrane 70 was purchased from Himedia Laboratories PVT. LTD. Soya phosphatidyl choline was purchased from Ottochemika, Mumbai, ethanol, propylene glycol, methanol, chloroform, isopropyl alcohol were purchased from Finar limited, Ahmedabad (Gujarat). All other chemicals used were of analytical grade.

METHODS
Drug-polymer interaction
FTIR (Fourier Transform Infrared Spectroscopy)
Infra red spectrum of any compound or drug gives information about the groups present in that particular compound. A spectrophotometer for recording the spectra in the infra red region consist of any optical system capable of providing the monochromatic light in the region from 4000 to 400 cm\(^{-1}\) and means of measuring the quotient of the intensity of the transmitted light and the incident light. To determine the drug-polymer interaction IR septra of physical mixture of drug and polymers in combination were taken.

Determination of \(\lambda\) max by UV-Spectroscopy
Clobetasol propionate was separately dissolved in methanol and methanolic phosphate buffer of (pH 5.5) and suitably diluted with respective solutions. Dilutions were scanned for absorption maxima in range of 200-400 nm.

Calibration Curve of Clobetasol propionate in Phosphate buffer (pH 5.5)
Clobetasol propionate (100 mg) was weighed and transferred into volumetric flask (100 ml). It was dissolved and diluted to volume with methanolic Phosphate Buffer (pH 5.5) to the concentration of 1000 µg/ml. Further the stock solution was then serially diluted with phosphate buffer (5.5) to prepare the different concentration 2, 4, 6, 8 up to 10 µg/ml of Clobetasol propinate. The absorbances of the solutions were measured against methanolic phosphate buffer (pH 5.5) as blank at 239 nm using UV spectrophotometer. The absorbance values were plotted against concentration (µg/ml) to obtain the standard calibration curve.
Calibration Curve of Clobetasol propionate in methanol
Clobetasol propionate (100 mg) was weighed and transferred into volumetric flask (100 ml). It was dissolved and diluted to volume with methanol to the concentration of 1000 µg/ml. Further stock solution was then serially diluted with distilled water to prepare the different concentration 5, 10, 15, 20, 25, upto 30 µg/ml of Clobetasol propionate. The absorbance of the solutions was measured against methanol as blank at 239 nm using UV spectrophotometer (Table 3.3). The absorbance values were plotted against concentration (µg/ml) to obtain the standard calibration curve (Figure 3.4).

Formulation of Clobetasol propionate loaded ethosomes by cold method
Clobetasol propionate (CP) loaded ethosomes were prepared by cold method followed by ultra sonicacion. Phospholipid, drug and other lipid materials were dissolved in ethanol in a covered beaker at room temperature by vigorous stirring with the use of magnetic stirrer. Propylene glycol was added during stirring. This mixture was heated to 30°C in a water bath. The water heated to 30°C in a separate beaker was added to the mixture, which was then stirred for 5 min in a covered vessel using magnetic stirrer. The vesicle size of ethosomal formulation was then reduced by ultrasonic probe sonicator for 10 min and off time was 10 sec. Finally, the formulation was stored under refrigeration.

Characterization of Ethosomes of Clobetasol Propionate
1. Clarity
Clarity was determined under fluorescent light against a white and black back ground for presence of any particulate matter.

2. pH
The pH of the prepared formulations after addition of all the ingredients was measured by using digital pH meter.

3. Vesicle shape
Ethosomes can be easily visualized by using transmission electron microscopy (TEM).

4. Vesicle size and zeta potential
Vesicle size of the ethosomes can be determined by Malvern instrument. Zeta potential of the formulation can be measured by Malvern zeta sizer.
5. **Drug entrapment**
The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique. The entrapment efficiency (EE), of Clobetasol propionate (CP) ethosomes, was determined by measuring the concentration of free Clobetasol propionate in the dispersion medium. 1.0 ml of the ethosomes was diluted up to 10 ml with phosphate buffer (pH 5.5) and centrifuged at 14000 rpm for 1 hour to separate the lipid and aqueous phase. Supernatant was than filtered by 0.2µ membrane filter and analyzed by UV-VIS spectroscopy at 239 nm.

\[
\text{% EE} = \frac{[\text{Initial drug} - \text{Free drug}]}{\text{Initial drug}} \times 100
\]

**Where,**

Initial drug = mass of initial drug used for the assay.

Free drug = mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

6. **Drug content**
Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.

Ethosomal suspension (1 ml) was taken and dissolved in 100ml methanol and shaken vigoursly for 2 hr in magnetic stirrer, there after the resultant solution was filtered and 1ml of sample was withdrawn and diluted to 10 ml in a volumetric flask and the absorbance was recorded in UV spectrophotometer at 239 nm.

7. **Stability studies**
The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by dynamic light scattering (DLS) and structure changes are observed by transmission electron microscopy (TEM).

8. **In-vitro release studies**
*In-vitro* drug release from the formulation was studied by the diffusion cell. Phosphate buffer pH (5.5) is used as diffusion medium, stirred at 50 rpm at 37ºC ± 0.5ºc. The 5 ml of ethosomes formulations were filled in test tubes and the end of the test tubes was covered with a dialysis membrane and was placed in such way that it touches the surface of diffusion medium present in receptor compartment. The drug sample were withdrawn at the interval of
half an hour for the time period of 12 hrs from diffusion medium and analyzed by using UV spectrophotometer at 239 nm, using phosphate buffer pH (5.5) as a blank.

RESULT AND DISCUSSION

**FTIR (Fourier Transform Infrared Spectroscopy)**

A spectrophotometer for recording the spectra in the infra red region consist of any optical system capable of providing the monochromatic light in the region from 4000 to 400 cm\(^{-1}\) and means of measuring the quotient of the intensity of the transmitted light and the incident light. The IR spectra indicates there are no interactions between drug and excipient.

**Determination of \(\lambda\) max by UV-Spectroscopy**

A solution of 100 \(\mu\)g/ml of drug was scanned in the range of 200 to 800 nm. From the scanning of drug in methanol and phosphate buffer it was concluded that the \(\lambda\) max of drug was found to be 239 which is same as reported.

**Calibration Curve of Clobetasol propionate**

From the standard curve of methanol and phosphate buffer pH 5.5 it was observed that the drug obeys Beer-Lambert’s law in concentration range of 1-30 \(\mu\)g/ml in the medium as shown in table. 2 and 3 and figure 6 and 7.

**Vesicle size and zeta potential**

Vesicle size and zeta potential of the ethosomes was determined by dynamic light scattering (DLS) by using Malvern Zeta meter. The final results of optimized batch are shown in table 4.

**Drug content and % entrapment efficiency**

The drug content and entrapment efficiency of the formulation are shown in table 5.

### Table 1: Composition of Ethosomes of Clobetasol propionate.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
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<tbody>
<tr>
<td>1.</td>
<td>Clobetasol propionate (mg)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
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<tr>
<td>2.</td>
<td>Soya phosphatidylcholine (mg)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>45</td>
<td>45</td>
<td>60</td>
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<tr>
<td>3.</td>
<td>Cholesterol (mg)</td>
<td>15</td>
<td>30</td>
<td>45</td>
<td>15</td>
<td>30</td>
<td>45</td>
<td>15</td>
<td>45</td>
<td>15</td>
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<tr>
<td>5.</td>
<td>Ethanol (ml)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>6.</td>
<td>Propylene glycol (ml)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<tr>
<td>7.</td>
<td>Distilled water (ml)</td>
<td>Up to 30 ml</td>
<td>Up to 30 ml</td>
<td>Up to 30 ml</td>
<td>Up to 30 ml</td>
<td>Up to 30 ml</td>
<td>Up to 30 ml</td>
<td>Up to 30 ml</td>
<td>Up to 30 ml</td>
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</tr>
</tbody>
</table>
Table 2: Data of Calibration Curve of Clobetasol propionate in Phosphate Buffer (pH 5.5).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance±S.D</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.247 ± 0.001</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.509 ± 0.001528</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.753 ± 0.001528</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.942 ± 0.001528</td>
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<tr>
<td>5</td>
<td>10</td>
<td>1.223 ± 0.001528</td>
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Table 3: Data of Calibration Curve of Clobetasol propionate in Methanol.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
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<td>0.178 ± 0.001</td>
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<tr>
<td>2</td>
<td>10</td>
<td>0.314 ± 0.001155</td>
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<td>3</td>
<td>15</td>
<td>0.460 ± 0.002</td>
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<td>4</td>
<td>20</td>
<td>0.606 ± 0.001528</td>
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<tr>
<td>5</td>
<td>25</td>
<td>0.732 ± 0.001</td>
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<td>6</td>
<td>30</td>
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Table 4: Zeta potential and particle size values of optimized formulation.

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<th>Formulation code</th>
<th>Particle size</th>
<th>PDI</th>
<th>Zeta Potential</th>
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<td>123.1</td>
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Table 5: Entrapment efficiency and zeta potential.

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<th>pH</th>
<th>% EE</th>
<th>% Drug content</th>
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<td>F2</td>
<td>5.62</td>
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<td>F3</td>
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<td>4</td>
<td>F4</td>
<td>5.51</td>
<td>67</td>
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<td>96.55</td>
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Table 6: Percentage Cumulative Release of Clobetasol Propionate Loaded Ethosomes.

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Figure 1: FTIR Spectra of Clobetasol propionate.

Figure 2: FTIR Spectra of Clobetasol propionate + Soya phosphatidylcholine.

Figure 3: FTIR Spectra of Clobetasol propionate + Cholesterol.
Figure 4: UV absorption spectrum of Clobetasol propionate in phosphate buffer pH 5.5.

Figure 5: UV absorption spectrum of Clobetasol Propionate in methanol.

Figure 6: Calibration curve of Clobetasol propionate in Phosphate Buffer (pH 5.5).
Figure 7: Calibration curve of Clobetasol propionate in Methanol.

Figure 8: *In-vitro* release of ethosomes formulation.

Figure 8: *In-vitro* release of ethosomes formulation.
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It’s my sincere privilege to express my thanks to my esteemed research guide Dr. Dinesh Kumar Sharma, Devsthali Vidyapeeth College of Pharmacy, Rudrapur. It gives me great pleasure to acknowledge my immense respect and gratitude to Mr. Arun kumar Singh (HOD) for the facilities provided. I sincerely thank Devsthali Vidyapeeth Collage of Pharmacy for providing me all the facilities for my research work.

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