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FORMULATION DEVELOPMENT AND EVALUATION OF ADAPALENE CONTAINING ETHOSOMAL GEL FOR TREATMENT OF ACNE

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Abstract:
In this study an attempt has been made to formulate a supplement dermal therapy of Adapalene. The ethosomal encapsulation of Adapalene was found to increase the skin residence time leading to a faster healing of external lesions and to a reduction of side effects and duration of therapy. Adapalene ethosomes prepared by hot technique with required modifications after optimizing formulation variables. Ethosomes were prepared and optimized on the basis of average vesicle size, and % drug entrapment. The optimized formulation was further encorpoated with gel base (carbapol gel) and characterized for their viscosity, extrudability, spreadability and drug release study. It was found that in all formulation formulation F2 select as a optimized formulation because of its good Spreadability (13.25±1.1), Viscosity (3215) and pH (66.80). The maximum % assay was also found in formulation F2 (95.21±0.15). In vitro drug release from ethosomal gel was carried out using Frenze diffusion cell method and found 96.75% in 12 hr. In first 30 min it was 25.65% drug release which slightly high. It was due to the release of free drug present in bag after leaching from liposomes. Drug release from ethosomes formulation was found in very sustained and controlled manner.

Keywords: *Adapalene, Ethosomal, Acne, Formulation, Evaluation*

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INTRODUCTION:

Acne is considered as one of the most widespread skin diseases. When extreme disfiguration occurs, it results in the development of severe consequences among the young people and may result in depression and suicide. *Acne vulgaris* is the second uppermost reason of suicide among skin diseases. When a person suffering from acne is compared with an individual who is not suffering from acne than it is found that the former has higher level of anxiety, more socio inhibition and has more aggressiveness [1]. Acne is an exclusive disease associated with skin occurs when sebaceous glands (SGs) attain special conditions. This disease occurs in both male and female; there is no preference among them, but the course is more severe in males [2].

Optimum therapeutic outcomes require not only proper drug selection but also effective drug delivery. The human skin is a readily accessible surface for drug delivery. Over the past three decades, developing controlled drug delivery has become increasingly important in the pharmaceutical industry. The pharmacological response, both the desired therapeutic effect and the undesired adverse effect, of a drug is dependent on the concentration of the drug at the site of action, which in turn depends upon the dosage form and the extent of absorption of the drug at the site of action [3].

Skin of an average adult body covers a surface of approximately 2 m 2 and receives about one-third of the blood circulating through the body. Skin contains an uppermost layer, epidermis which has morphologically distinct regions; basal layer, spiny layer, stratum granulosum and upper most stratum corneum, it consists of highly cornified (dead) cells a continuous matrix of lipid membranous sheets. These extracellular membranes are unique in their compositions and are composed of ceramides, cholesterol and free fatty acids [4].

The human skin surface is known to contain, on an average, 10-70 hair follicles and 200-250 sweat ducts on every square centimeters of the skin area. It is one of the most readily accessible organs of the human body. The potential of using the intact skin as the port of drug administration to the human body has been recognized for several decades, but skin is a very difficult barrier to the ingress of materials allowing only small quantities of a drug to penetrate over a period of time. Transdermal drug delivery-the delivery of drugs across the skin and into systemic circulation - is distinct from topical drug penetration which targets local areas. Transdermal drug delivery takes advantage of the relative accessibility of the skin [5].

Chemically, adapalene (ADP) is 6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoic acid. It is a naphthoic acid derivative and retinoid analogue with actions similar to those of tretinoin. It is used in topical treatment of mild to moderate acne

Therefore, reliable drug delivery systems providing better drug penetration can result in better efficacy and also help in the prevention of development of resistance. The aim of the present study was to statistically optimize the ethosomal gel for enhanced skin delivery of Adapalene which was effective candidate for the treatment of acne.

MATERIAL AND METHODS:

Preparation of Ethosomes of Adapalene:

Soya PC (0.5 to 1.5% w/v) was dissolved in ethanol (10-20% v/v) and heated up to 30 ± 1°C in a water bath in a closed vessel. Distilled water or drug solution in distilled water (0.1% w/v solution), which is previously heated up to 30 ± 1°C, was added slowly in a fine stream to the above ethanolic lipid solution with continuous mixing using a magnetic stirrer at 900 rpm. Mixing was continued for another 5 minutes and finally, the vesicular dispersions resulted was left to cool at room temperature (25 ± 1°C) for 45 minutes [6]. Different ethosomal dispersions and their composition are shown in table 1.

Table 1: Different Composition of ethosomes formulation

F. Code	Drug (mg)	Phospholipid (% w/v)	Ethanol (% w/v)	PEG (%w/v)	Water (%w/v)
F1	100	0.5	10	20	100
F2	100	0.5	20	20	100
F3	100	1.0	10	20	100
F4	100	1.0	20	20	100
F5	100	1.5	10	20	100
F6	100	1.5	20	20	100

Formulation of ethosomal gel:
The incorporation of the drug loaded ethosomes (equivalent to 0.1%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes. The optimized formulation was incorporated into three different carbapol gel concentration 0.5, 1 and 2% w/w [7]. Table 2.

Table 2: Composition of different gel base

S. No.	Formulation	Carbapol (%)
1.	F1	0.5
2.	F2	1
3.	F3	2

Evaluation of Adapalene loaded Ethosomes:
Vesicle size and zeta potential:
Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy [8] using a horiba scientific, nanoparticle analyzer instrument the results shown in table 3.

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded ethosomes dispersion was placed in the eppendorf tubes and centrifuged at 10,000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain ethosomes without Adapalene was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 238nm [9]. The results shown in table 3.

Formulation of ethosomal gel:
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Evaluation of gel:
Physical Characteristic:
The Physical Characteristic was checked for gel formulations (homogeneity and texture) and observations were shown in Table 4.
Determination of pH:
The pH of the gel was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted Shah et al., [11]. The measurements of pH of each formulation were replicated two times (table 4).
Washability:
Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations were shown in Table 4.

Extrudability study:
The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked in table 7.5.
Assay:
Weight equivalent to 10 mg of ethosomal gel dissolved in 5 ml methanol in 10 ml volumetric flask, sonicate it for 10 min and volume make up to 10 ml and dilute suitably to 10µg/ml and take the absorbance at 238 nm and calculate using calibration curve of linearity.
Spreadability:
An important criterion for gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application area. The therapeutic efficacy of a formulation also depends on its spreading value.
A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip of from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability.

Method:
Two glass slides of standard dimensions (6×2) were selected. The gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the gel formulation between the two slides was traced uniformly to form a thin layer.

The weight was removed and the excess of the gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50 with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and

separate away from lower slide under the direction of the weight was noted [12]. The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation (Table 5).

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)
m = weight tied to the upper slide (20 grams)
l= length of glass slide (6cms).
t = time taken is seconds.

Viscosity:
The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25^0C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25±/1^0C) before the measurements (Table 6).

***In-vitro* drug release studies using the semipermeable membrane Preparation of semipermeable membrane for the diffusion studies:**
The semipermeable membrane approximately 25 cm x 2cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies.

The prepared Ethosomes delivery system was evaluated for *in vitro* drug release. The drug release studies were carried out using modified franz diffusion cell. The dissolution study was carried out in 24 ml dissolution medium which was stirred at 50 rpm maintained at 37±0.2°C.

Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 238nm for Adapalene and using UV visible spectrophotometer. The release of Adapalene was calculated with the help of Standard curve of Adapalene [13].

The observations of drug release for the drug in uncoated formulation and coated formulation is tabulated in Table 6.

RESULTS AND DISCUSSION:

In this study an attempt has been made to formulate a supplement dermal therapy of Adapalene. The ethosomal encapsulation of Adapalene was found to increase the skin residence time leading to a faster healing of external lesions and to a reduction of side effects and duration of therapy. Adapalene ethosomes prepared by hot technique with required modifications after optimizing formulation variables.

Ethosomes were prepared and optimized on the basis of average vesicle size, and % drug entrapment. The optimized formulation was further encorpoated with gel base (carbapol gel) and characterized for their viscosity, extrudability, spreadability and drug release study. It was found that In all formulation formulation F2 select as a optimized formulation because of its good Spreadability (13.25±1.1), Viscosity (3215) and pH (66.80). The maximum % assay was also found in formulation F2 (95.21±0.15). In vitro drug release from ethosomal gel was carried out using Frenze diffusion cell method and found 96.75% in 12 hr. In first 30 min it was 25.65% drug release which slightly high. It was due to the release of free drug present in bag after leaching from liposomes. Drug release from ethosomes formulation was found in very sustained and controlled manner.

Table 2: Result for Vesicle size and Entrapment efficiency of drug loaded Ethosomes

Formulation Code	Vesicle size (µ)	% Entrapment Efficiency
F1	185.65±0.25	71.12±0.32
F2	192.24±0.15	73.32±0.25
F3	165.45±0.36	69.98±0.14
F4	158.85±0.41	68.85±0.36
F5	110.25±0.32	86.65±0.47
F6	130.26±0.18	73.32±0.36

Table 3: Vesicle size and entrapment efficiency of optimized ethosomes

Formulation Code	Vesicle size (nm)	Entrapment Efficiency	Zeta potential
F5	110.25±0.32	86.65±0.47	-38.5

Table 4: Results of Homogeneity, Extrudability, Spreadability of gel formulation

Code	Homogeneity and Texture	Spreadability (gm.cm/sec.)	Extrudability	Washability
EF1	+++	16.65±1.2	+++	**Good**
EF2	+++	13.25±1.1	+++	**Good**
EF3	+++	11.45±0.8	+++	**Good**

+++ Good **++ Average**

Table 5: Results of pH, Viscosity and % Assay

Code	pH	Viscosity (cps)	% Assay
EF1	6.74	3565	92.25
EF2	6.80	3215	98.85
EF3	6.95	3025	94.45

Table 6: Cumulative % drug release of Adapalene from optimized ethosomes gel formulation F2

S. No.	Time (hrs)	% Cumulative drug release ethosomal gel
1	0.5	25.65
2	1	42.32
3	2	56.65
4	4	68.81
5	6	73.32
6	8	85.65
8	10	96.75

CONCLUSION:

It was concluded that prepared gel containing Adapalene loaded ethosomal formulation was optimized and successfully formulated in the form gel can be use for topical preparation for antiacne affect.

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