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FORMULATION, DEVELOPMENT AND EVALUATION OF ETHOSOMAL GEL OF CLINDAMYCIN PHOSPHATE FOR ACNE VULGARIS

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ABSTRACT

The objective of the research work was in aim to formulate and evaluation of ethosomes containing clindamycin phosphate by using phospholipid (0.5-1%), ethanol (10-30%), propylene glycol (20%) and distilled water by cold method. Prepared ethosomal vesicles evaluated for vesicular size, shape, entrapment efficiency, skin irritation and stability studies were carried out. Scanning electron microscopy and size analyser results showed that ethosomes were in spherical, unilamellar, nanometric size. The formulation F4 showed highest entrapment efficiency of 82.36±0.32% and vesicle size was found to 110±0.14. Then, optimized formulation of ethosomal vesicles (F4) was further formulated to gel by using carbopol (0.5-2%) . EF2 was found to have shown excellent invitro drug release comparing gel containing free clindamycin drug and entrapment efficiency. Stability studies performed (EF2) at refrigeration temperature (4.0±0.2°C) and at room temperature (25-28±2°C) for 3 months. The kinetic study was found to be fit in zero order models and observed no remarkable symptoms on skin from skin irritation study. The present work also focuses on making the formulation more pharmaceutically acceptable.

K**eywords:** Clindamycin, Ethosomal gel, Phospholipid, Entrapment efficiency, stability studies

INTRODUCTION:

Topical drug delivery system is considered to be one of the most relevant routes for treating skin diseases efficaciously. Despite of having the advantages of self-administration, patient compliance and reduction in adverse effects systemically, this system has the limitation of slow diffusion across the stratum corneum[1] and the barrier property of the skin limits the delivery of the drug through the skin [2].Therefore several approaches have been used to weaken this skin barrier and to enhance the administration of the drug through the skin. One of these approaches is the use of vesicle formulations as skin dermal system[3]. The various vesicular systems investigated, ethosomal vesicles have been found to be capable enough in enhancing permeation of topical agents to the deeper tissues through the stratum corneum [4]. Enhanced permeation through these vesicles is not only due to the presence of ethanol but also due to the fact that these vesicles are highly deformable and malleable that allows their better penetration across the skin [5]. Ethosomes, soft vesicles consisting of biocompatible ingredients, phospholipids, ethanol and water, were introduced by Touitou [6, 7]. According to the suggested mechanism of skin permeation enhancement by ethosomes, ethanol fluidizes the lipid bilayers of the stratum corneum and of the ethosome, resulting in

a more penetrable skin and less rigid vesicle. Further the soft vesicle penetrates the disturbed skin bilayers and releases the drug in the skin layers along the penetration pathway. It was shown that ethosomes are efficient carriers for drugs within a wide range of physicochemical proprieties: lipophilic, ionic, polar and high molecular weight molecules [8-11]. Gel formulation is defined as a substantially diluted cross-linked system, which exhibits no flow when in the steady-state [12]. It is a dispersion of molecules of a liquid within a solid created by the cross-linking of each other to constitute a three-dimensional cross-linked network. In this way, gel formulation has properties between solid phase and liquid phase. Recently, gel formulation has become one of the most relevant routes for treating skin diseases efficaciously [13]. It is of practical significance using percutaneous drug delivery and transdermal absorption in clinics. Clindamycin phosphate is a semi synthetic antibiotic. Clindamycin inhibits bacterial protein synthesis at the level of the bacterial ribosome. The antibiotic binds preferentially to the 50S ribosomal subunit and affects the process of peptide chain initiation. Clindamycin is indicated for the treatment of bacterial vaginosis (formerly referred to as Hemophilus vaginitis, Gardnerella vaginitis, nonspecific vaginitis, corynebacterium vaginitis, or anaerobic vaginosis) in nonpregnant women [14]. Hence, in our present work, developing and optimizing a ethosomal gel system of clindamycin for topical application.

MATERIALS AND METHODS

Material

Phospholipids purchased from Himedia Laboratory, Mumbai. Ethanol, propylene glycol and carbopol-934 purchased from CDH chemical Pvt. Ltd. New Delhi. Dialysis membrane of Mol Wt cutoff 1200 was purchased from Himedia Laboratory, Mumbai. Double distilled water was prepared freshly and used whenever required. All other ingredients and chemicals used were of analytical grade.

Preparation of Ethosomes of Clindamycin

Soya PC (0.5 to 1% w/v) was dissolved in ethanol (10-30% 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. 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	0.5	30	20	100
F4	100	1.0	10	20	100
F5	100	1.0	20	20	100
F6	100	1.0	30	20	100

Preparation of ethosomal gel

The incorporation of the drug loaded ethosomes (equivalent to 1%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes. The optimized formulation (F4) was incorporated into three different carbapol gel concentration 0.5, 1 and 2% w/w table 2.

Table 2 Composition of different gel base

S. No.	Formulation	Carbapol (%)
1	**EF1**	0.5
2	**EF2**	**1**
3	**EF3**	**2**

RESULT AND DISCUSSION

Evaluation of ethosomes

Microscopic observation of prepared ethosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomes formulation. It was observed that ethosomes were in spherical, unilamellar, nanometric size.

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Results were shown in table 3. The vesicles size of all formulation was found to be in the range of 110±0.14 145±0.27nm. From the results it was concluded that formulation F4 has size 110±0.14nm was best formulation.

Zeta potential

The zeta potential was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm. The zeta potential of optimized formulation was -35mv.

Entrapment efficiency

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 Clindamycin 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 486 nm using Labindia 3000+ spectrophotometer [15]. Results were shown in table 3 and fig 1. From the result it was concluded that formulation F4 has maximum % EE 82.36±0.32.

$$\%\ \boldsymbol{Entrapment\ Efficiency} = \frac{\boldsymbol{Therotical\ drug\ content - Practical\ drug\ content}}{\textbf{Therotical drug content}} \times \mathbf{100}$$

Table 3 Result for Vesicle size and entrapment efficiency of drug loaded ethosomes

F. Code	Vesicle size (nm)	% EE
F1	125±0.12	69.12±0.15
F2	130±0.32	72.35±0.24
F3	145±0.27	70.42±0.25
F4	110±0.14	82.36±0.32
F5	125±0.25	69.45±0.26
F6	130±0.31	71.65±0.14

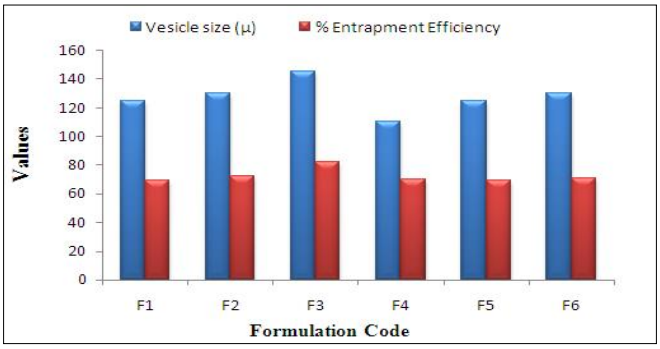

Fig. 1 Graphical representation of vesicle size and entrapment efficiency

Evaluation of ethosomes gel

The **physical** characteristic was checked for gel formulations (homogeneity and texture). From the result it was concluded that formulation EF1and EF2 has good homogeneity and texture. Formulations were applied on the skin and then ease and extent of washing with water were checked manually. 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. All the 3 formulation has good washability and extrudability. Results were given in table 4. 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. The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation. The spreadability of all formulation was found to be in range of 12.32±3.49 to16.65±1.45.

Table 4 Results of homogeneity, extrudability, spreadability of gel formulation

Code	Homogeneity and Texture	Spreadability (gm.cm/sec.)	Extrudability	Washability
EF1	+++	16.65±1.45	+++	**Good**
EF2	+++	14.45±2.32	+++	**Good**
EF3	++	12.32±3.49	+++	**Good**

+++ Good ++ Average

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. The measurements of pH of each formulation were replicated two times.The pH of all formulation was found in the range of 6.87±0.08 to7.12±0.12. 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. From the result it was concluded that

increase the concentration of gel increase viscosity. For determination of % 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 486 nm and calculate using calibration curve of linearity. Formulation EF2 has maximum drug content 95.21±0.15 table 4.

Table 4 Results of pH, Viscosity and % Assay

Code	pH	Viscosity (cps)	% Assay
EF1	7.12±0.12	2565	89.89±0.19
EF2	6.98±0.14	2872	95.21±0.15
EF3	6.87±0.08	3012	90.25±0.45

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 486 nm for Clindamycin and using UV visible spectrophotometer. The release of Clindamycin was calculated with the help of Standard curve of Clindamycin table 5.

Table 5 Cumulative % drug release of optimized ethosomes gel EF2

S. No.	Time (hrs)	% Cumulative Drug Release Ethosomal gel
1	0.5	34.45
2	1	38.89
3	2	45.65
4	4	55.56
5	6	65.45
6	8	88.89
8	10	94.56

The *in vitro* drug release data of the formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation and Korsmeyer's models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r^2' values of formulation was maximum i.e 0.981 hence indicating drug release from formulations was found to follow zero order drug release kinetics table 6,7 and fig. 2-4.

Table 6 *In Vitro* Drug Release Data for optimized gel formulation EF2

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release ± SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	0.5	0.707	-0.301	34.45±0.25	1.537	65.55	1.817
2	1	1	0	38.89±0.23	1.590	61.11	1.786
3	2	1.414	0.301	45.65±0.14	1.659	54.35	1.735
4	4	2	0.602	55.56±0.26	1.745	44.44	1.648
5	6	2.449	0.778	65.45±0.32	1.816	34.55	1.538
6	8	2.828	0.903	88.89±0.45	1.949	11.11	1.046
7	10	3.162	1	94.56±0.32	1.976	5.44	0.736

* Average of three determinations

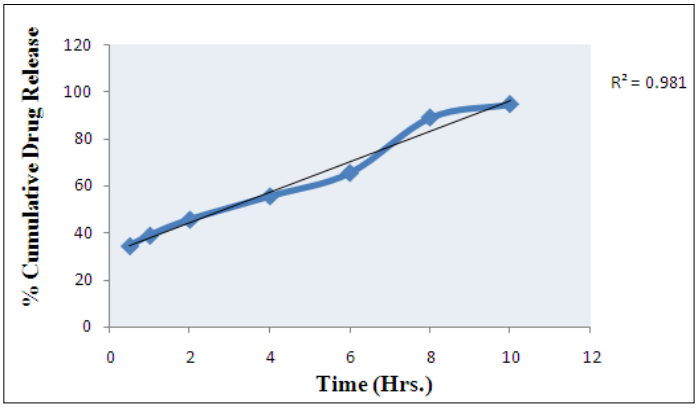

Fig. 2 Zero order release study

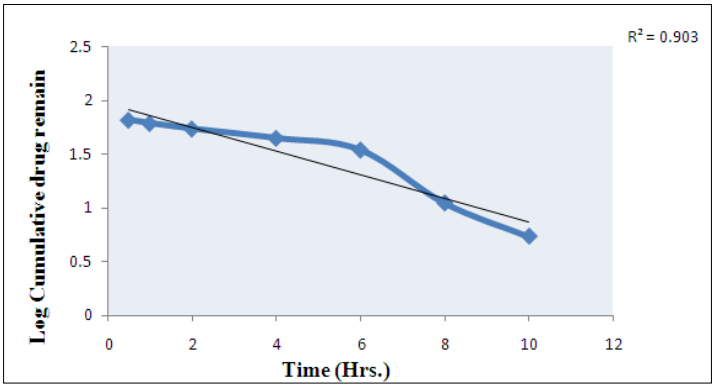

Fig. 3 First order release study

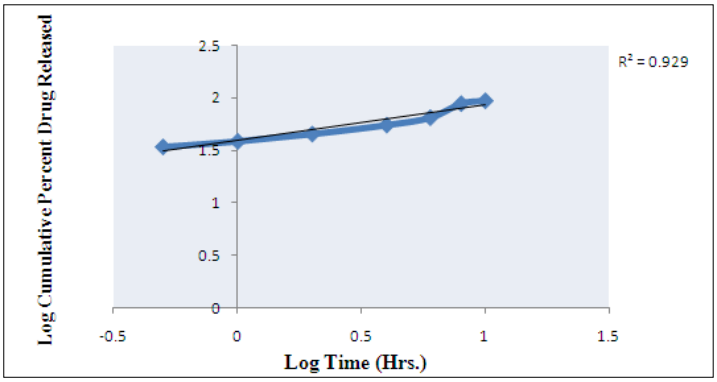

Fig. 4 pappas Release study

Table 7 Regression analysis data of ethosomal gel

Formulation	Zero order	First order	Pappas plot
EF2	0.981	0.903	0.929

Stability studies were carried out with optimized formulation which was stored for a period of 45 days at 4±1°C, RT and 40±1°C. The particle size of formulation was determined by optical microscopy using

a calibrated ocular micrometer. The vesicle size of the ethosomes was found to increase at RT, which may be attributed to the aggregation of ethosomes at higher temperature. At 45±2°C the aggregate i.e. these ethosomes were unstable at higher temperature like 45±2°C. Percent efficiency of ethosomes also decrease at higher temperature like 45±2°C.

CONCLUSION

In the present study, Clindamycin phosphate entrapped ethosomal gel for transdermal drug delivery was prepared by using various concentrations of phospholipids and ethanol. The prepared formulation showed good entrapment efficiency, particle size and drug release. From the results obtained, the formulation EF2 is concluded as the best formulation, spherical vesicular shape and 95.21% drug entrapment efficiency. It was observed that EF2 was follow zero order drug release kinetics it can be suggested that ethosomes could be superior drug carrier for topical delivery of clindamycin phosphate. In future research, preclinical studies of the optimized formulation are to be performed.

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