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FORMULATION & EVALUATION OF ETHOSOMAL PATCH FOR DELIVERY OF METHOXSALENE

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ABSTRACT

Transdermal drug delivery system minimizes the systemic absorption thereby increases the bioavailability. Methoxsalene is an antiplatlet drug generally avelable in tablet formulation. Oral delivery of Methoxsalene leads to its metabolism by cyp450 resulting in its active metabolite R-130964 and inactive metabolite. All the systems were characterized for vesicle morphology, particle size and entrapment efficiency by Scanning Electron Microscopy, Transmission Electron Microscopy, Differential light scattering and centrifugation respectively. The effect of different formulation variable on skin permeation of Methoxsalene was studied via synthetic semi permeable membrane by using diffusion cell. Methoxsalene entrapped ethosomal vesicles were prepared by hot method.

Keywords: Transdermal drug delivery, ethosomes, Methoxsalene, novel drug delivery system.

INTRODUCTION

Transdermal drug delivery system minimizes the systemic absorption thereby increases the bioavailability. Methoxsalene is an antiplatlet drug generally avelable in tablet formulation. Oral delivery of Methoxsalene leads to its metabolism by cyp450 resulting in its active metabolite R-130964 and inactive metabolite. But the percentage of inactive metabolite formed (85%) is more than that of its active metabolite (15%).thus to maintain proper loading dose Methoxsalene (75mg) has to be taken frequently with 50% bioavailability. Thus formulation of Methoxsalene ethosomal transdermal patch is done because formation of ethosomes leads to the effective absorption of drug through skin. It also increases the penetaration power of drug [1-3]. Methoxsalene through skin gets metabolized by cyp-2c19 into its active metabolite. Ethosomes have higher penetration rate through the skin as compared to liposomes hence these can be widely used in place of liposomes[4]. The increased permeation of ethosomes is

probably due to its ethanolic content. Ethanol increases the cell membrane lipid fluidity which results in increased skin penetrability of ethosomes [5-6].

The purpose of this research work was to formulation and evaluation of transdermal drug delivery system of Methoxsalene using various polymers such as carbapol, propylene glycol, lecithin by solvent evaporation technique for improvement of bioavailability of drug and reducing toxic effects.the presence of ethanol increases the flaxivility of the lipid bilayer him ethosomes [6-8].

Material and Method:

Material:

Methoxsalene was kindly gift sample on IPCA Pharmaceutical Pvt ltd.. Triethylcitrate Phospholipid, Acrylic resin Carbapol, obtained from Himedia Laboratory. Other chemical was purchased from local market AR grade.

Method:

Preparation of Methoxsalene loaded ethosomal vesicles:

FORMULATION OF ETHOSOMES

The ethosomal system of Methoxsalene comprised of 1.0% w/w drug, 1-4.0% phospholipids, 5% propylene glycol, 10-20 % ethanol and aqueous phase to 100% w/w.

The drug was then dissolved in methanol to obtain a concentration of 1.0% w/w. From the above stock solution 5ml were diluted with PBS buffer pH 7.4. To warm water (30°C) the phospholipids were dispersed by heating on a water bath to get a colloidal solution. To a separate flask ethanol and PEG were mixed and heated to temperature of 30°C. Once both mixtures reached 30°C, the aqueous phase was added to the organic mixture with stirring at 700 rpm. Subsequent to mixing, stirring was continuous for a further 5 minutes. Temperature was maintained at 30°C during the entire process. The ethosomes prepared were subjected to sonication at 4^0 C in3 cycles of 10 minutes with 5 minutes rest between the cycles [6-10].

Table 1. Formulation composition for ethosomes

S.no.	Formulation Code	Phospholipid (% w/w)	Ethanol (% w/w)	Drug (% w/w)
1	MET1	2	10	1
2	MET2	3	15	1
3	MET3	4	20	1

FORMUTATION OF ETHOSOMAL TRANSDERMAL PATCH

Transdermal patch of ethosomal formulation containing 20% ethanol and patch containing pure Methoxsalene was prepared for comparative drug release study. The casting solution was prepared by dissolving weighed quantities of PVP and PVA in water by heating on a water bath at 70^0C. To the resulting solution PEG-4000 was then added to the and cooled. Then required quantity of drug or ethosomal formulation was added and mixed thoroughly to form a homogeneous mixture. The volume was made up to 6 ml with purified water [11-12].

The casting solution was then poured into glass mould specially designed to seize the contents. To a glass mould contains the casting solution was dried upto 24 hrs at room temperature in vacuum oven. The patch was removed by peeling and cut into round shape of 1 cm^2. These patches were kept in desiccators for 2 days for further drying and enclose in aluminium foil and then packed in self- sealing cover.

Table 2. Formulation composition of ethosomes loaded TDP

Formulation Code	PVP (mg)	PVA (mg)	PEG-4000 (mg)	Drug	Water
TDP1	125	75	250	3ml of ethosomes	Upto 6 ml
TDP2	125	75	250	75 mg	Upto 6 ml

Evaluation of Ethosomes

Particle size and shape analysis

Microscopic analysis was performed to measure the average size of formulated ethosome. Then formulated ethosomes were diluted with distilled water to facilitate observance of individual vesicle and a drop of diluted suspension was taken on a glass slide covered with cover slip and examined under trinocular microscopic (magnification 400X). The diameters of 150 vesicles were determined at random using calibrated eyepiece micrometer with stage micrometer. The average diameter was calculated using the formula [12-14].

$$\textbf{Average diameter}=\frac{\Sigma n.d}{\Sigma n}$$

Where n = number of vesicles; d = diameter of the vesicles

By means of sonication vesicular size is reduced since these vesicles could not be analysed under microscope at 15 × 45 X. magnification. Therefore, the ethosomes of observed through transmission electron microscope.

TEM (Transmission electron microscopy)

The surface morphology of selected ethosomal formulation was evaluated by the transmission electron microscopy, TEM (Punjab Technical University). In order to perform the TEM observation the diluted ethosomal sample were placed on the holey film grid (copper film grid) and observed after drying of the sample.

Entrapment efficiency

The entrapment efficiency of methoxsalene in ethosomal vesicle were estimated by ultracentrifugation technique, 10ml of ethosomal formulation were mixed with 1 ml of 1 % triton X-100 solution. Each sample was vortexed for 2 cycles of 5 min with 2 minutes rest between the cycles. Specified quantity 1.5ml of vortexed sample and fresh untreated ethosomal formulations were taken into different centrifugal tubes. The samples were allowed centrifugation 20,000 rpm for 3 hours. From the mixture the supernatant were separated, diluted with water suitably and drug concentration was determined at 239 nm in both vortexed and unvortexed samples. The entrapment efficiency was calculated as follows[15-16]:

$$\textbf{Entrapment Efficiency} = \frac{T-C}{T} \times 100$$

Where.

'T' = total amount of drug that detected from supernatant of vortexed sample

'C' = the amount of drug unentraped and detected from supernatant of unvortexed sample.

pH measurements

pH of selected formulations were determined with the help of digital pH meter (Jyoti labo). Using a calibrated pH meter for measurement the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and reading shown on display get noted [14-18].

Evaluation of Ethosomal Patch

Physiochemical evaluation

Thickness

Take randomly thickness using screw gage at various locations on Matrix film. The value reported is mean of three consecutive readings.

Uniformity of weight

Determine the weights of randomly selected patches prepared in batches. The value reported is mean of three consecutive readings.

Drug content

Weigh accurately portion of film (100 mg) and dissolved it in 100 ml of ethanol (95%), then shake the resulting mixture vigorously to dissolve. Withdraw the sample filter it and scanned at wavelength of drug using UV spectrophotometer.

Moisture content

Individually weighted films and kept them in desiccator containing calcium chloride at room temperature for at least 24 hrs to check the moisture uptake by formulated patches [17-20].

Now weigh the films again; the difference in weight (initial and final weight) gives moisture content.

$$\% \text{ Moisture content} = \frac{\text{Initial weight- final weight}}{\text{Initial weight}} \times 100$$

Moisture uptake

Weigh individually the films and kept them in desiccator containing calcium chloride at room temperature for at least 24 hrs. remove the films from desiccators and exposed to 4Rh using saturated solution of KCl in a another desiccators till uniform weight is achieved.

$$\% \text{ Moisture uptake} = \frac{\text{Final weight – Initial weight}}{\text{Final weight}} \times 100$$

Drug entrapment study

The total drug entrapped in formulated ethosomal patch was carried out for a period of four weeks. Regular samples were taken at 2 weeks interval and study was performed by ultra centrifugation [6].

***In-vitro* Diffusion Study**

The *in-vitro* diffusion study taken by Franz Diffusion Cell. For this purpose Egg membrane was taken as semi permeable membrane in Franz diffusion cell receptor compartment (vol approx 60 ml) with effective surface area for permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A 2 cm^2 size patch was taken and placed on one side of membrane towards donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is maintained by water jacket in order to maintain the temperature of 37 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell [7-9]. After each sampling equal volumes of fresh receptor fluid was replaced in order to maintain sink condition then analyzed spectrophotometrically.

Stability studies

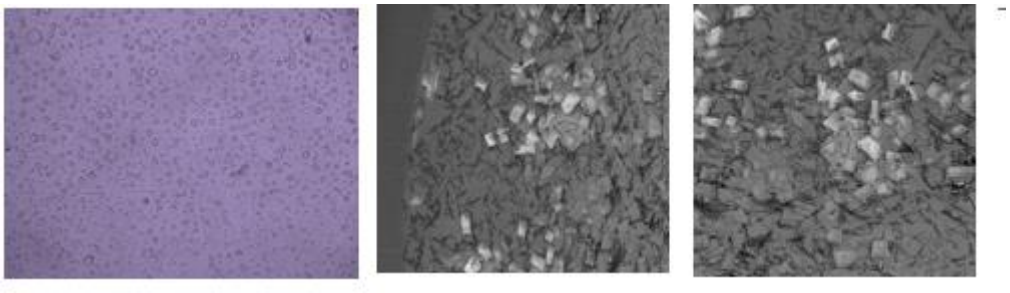
Stability study was carried out for methoxsalene ethosomal patch at two different temperatures i.e. refrigeration temperature (4 ± 2 °C) and at room temperature (25 -28±2°C) for 4 weeks. The ethosomal

patch were analysed for any physical changes such as color and appearance, entrapment study and drug content [11-13].

RESULT AND DISCUSSION:

Evaluation of Ethosomes

Particle size and shape analysis



(a) (b) (c)

Figure 1. Particle size and shape analysis of formulations

Table. 3: Size distribution of methoxsalene ethosomes

Size range Eye piece micrometer division	In micrometer	Average size (d)	No. of vesicles (n)*	% No. of vesicles	n×d
0-1	0.00-2.83	1.14	98	62.33	111.72
1-2	2.83-5.56	3.21	32	24.66	102.72
2-3	5.56-7.99	7.32	6	426	43.92
3-4	7.99-11.32	9.86	8	5.34	78.88
4-5	11.32-13.50	11.78	0	0	0
			∑n=144		**∑nd=337.24**

*Each value is an average of 3 replications.

$$\text{Average diameter} = \Sigma \frac{n.d}{\Sigma n}$$

$$= \frac{337.24}{144} = 2.341\mu m$$

Formation of multilamellar vesicles was confirmed by examining the ethosomal dispersion under an trinocular microscope (magnification 400X). The study reveals the predominance of spherical shaped vesicles. The vesicles of size and are appeared in multilayered. Visual observation of ethosomes confirmed the multi-lamellar vesicular structure; this showed presence of vesicles in ethosomes

regardless of having high concentrations of ethanol. The Methoxsalene ethosomal formulation CET3 with 20% ethanol and 4% phospholipids the average diameter was found to be 2.341 μm.

Figure.2. Formulated ethosomes

TEM (Transmission electron microscopy)

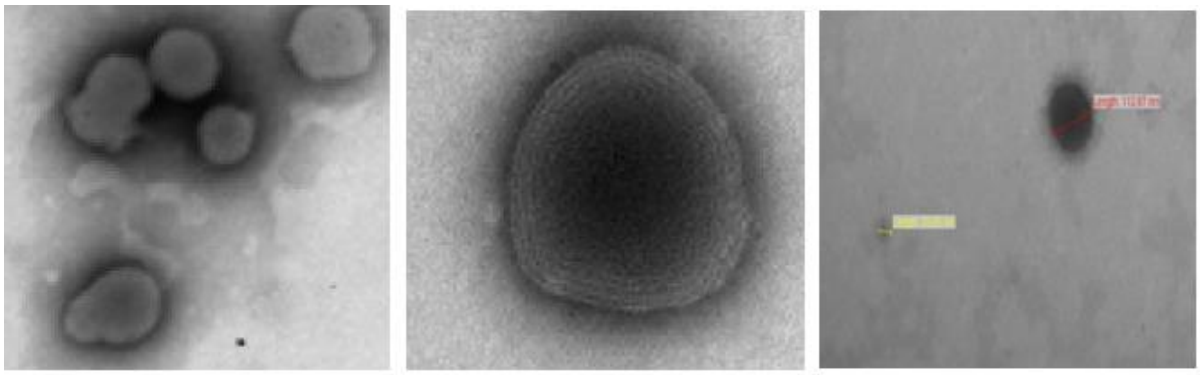


Figure.3. TEM results of prepared ethosomes

The study reeled that formulated ethosomes are having the diameter of 372.02nm, 136.05 nm, 114.87 nm and 31.65 nm this thus the etosomal vesicles can easily cross the barriers of body ethanol facilitates the permeation of the ethosomes across the menbranes.

Entrapment efficiency

Using ultracentrifugation method ethosomal vesicles containing drug and un-entrapped or free drug were separated to find out the entrapment efficiency. Results obtained are given in table 4.

Table 4. Drug Entrapment efficiency of ethosomes

Sample		Abs*	Concentration C (µg/ml)	Amount of drug CxDF(µg)	Entrapped drug E=T–U(µg)	% Drug entrapped % =E/Tx100
CET1	Total drug(T)	0.2535	9.602	1920.46	550.2	29.59%
	Free durg	0.1822	6.851	1370.26		
CET2	Total drug(T)	0.2543	9.633	1926.64	1020.6	52.31%
	Free durg	0.1221	4.530	906.04		
CET3	Total drug(T)	0.2604	9.871	1973.74	1429.6	710.84%
	Free durg	0.0752	2.721	544.14		

From the data obtained, the maximum entrapment efficiency of ethosomal vesicle as determined by ultracentrifugation was 71.48±0.89% for CET3 formulation containing 20% ethanol concentration. This was much higher than ethosomal formulation CET2 with 15% ethanol. As the ethanol concentration increased from 10% to 20% there was improvement in entrapment efficiency. The formulation CET1 with 10% ethanol concentration was having entrapment efficiency of 29.59±0.67%, beyond 20% ethanol concentration the entrapment efficiency was found to be at optimum level.

Vesicular Size and Surface Charge

The results of zeta potential shows that particles of ethosomes have zeta potential of -17.4mV and Electrophoretic mobility mean was found to be -0.000135cm^2 VS.

pH measurements

pH meter was used to measure the pH of ethosomal vesicles. The pH of the vesicles was fall within pH 4.0-5.0. The result of pH revealed that the pH increases as the ethanol concentration increased when the phospholipid concentration was constant. However, the pH decreases as the phospholipid concentration increase when the ethanol concentration was constant.

Evaluation of Ethosomal Patch

Physiochemical evaluation

Thickness

The thickness of prepared liposomal patch was found to be 0.80 mm thick, showing uniform thickness throughout the distribution which confirms uniform dispersion of ethosomes in prepared patch.

In-vitro Diffusion Study:

The objectives in the developments of in-vitro diffusion study are to show the release rates and extent of drug release from dosage form. The study was carried out for 72 hours duration, all results were shown on table and represented graphically. For the formulation CET3 with 20% ethanol concentration, the percentage drug release after one hour was only 1.45±0.04%, which was improved to 22.39±0.17% after 4 hours of diffusion. The percentage drug release from CET1 was almost doubled to 44.40±0.51% after 12 hours of study. After 24 hours of diffusion, the percentage drug release was found to be 55.57±0.60%. There was an increase of nearly 11 % in drug release during the twelve-hour duration. The percentage drug release on 48th, 60th hours of study was 65.85±0.25% and 71.04±0.22% respectively for CET1. At the end of diffusion study after 72 hours, the percentage drug release from CET1 was 78.03±0.18% and 21.97% was remaining within the CET1.

Table .5: *In-vitro* Diffusion Study for Ethosomal patch

Time in Hours	Dilution factor	% Drug unreleased	% Drug released*	± SD
0	10	100	0	0
1	10	97.97	2.03	0.04
2	10	85.4	14.6	0.12
4	10	75.35	24.65	0.17
6	10	61.4	38.6	0.15
12	10	55.1	44.9	0.51
24	10	41.1	58.9	0.6
36	10	37.7	62.3	0.25
48	10	28.7	71.3	0.25
60	10	22.5	77.5	0.22
72	10	15.5	84.5	0.18

**Values are represented as mean ±SD (n=6)*

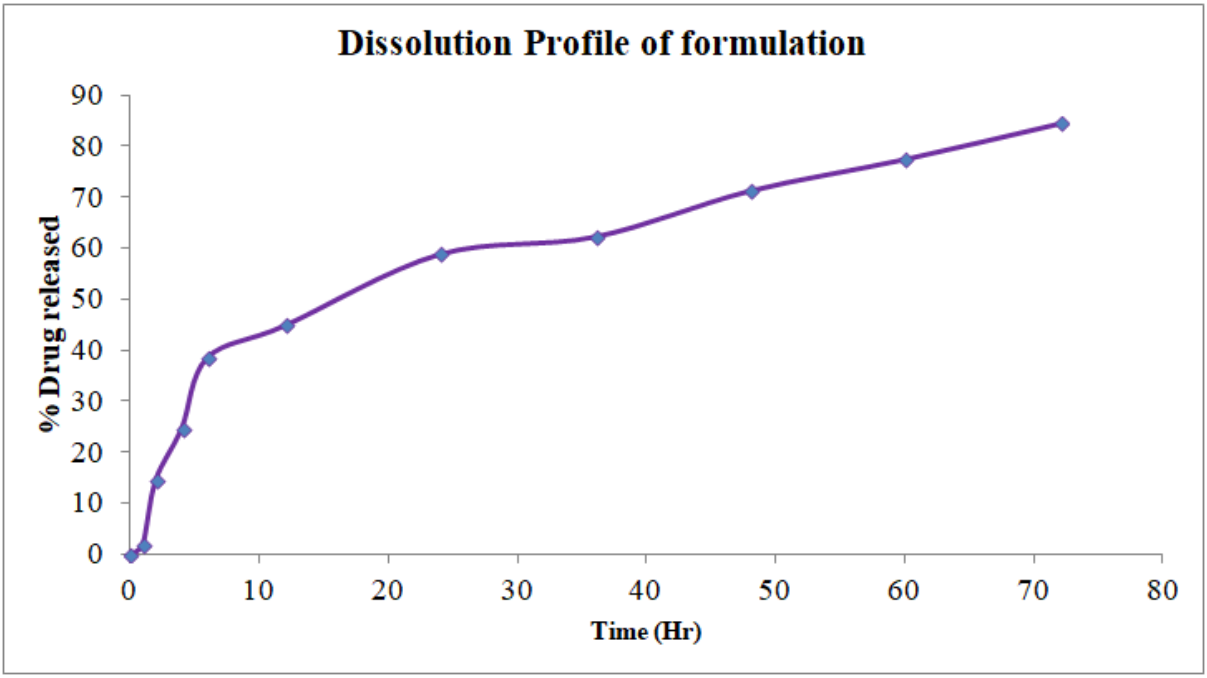


Figure 4. *In-vitro* Diffusion Study for Ethosomal patch

Stability studies

Ethosomal patch preparations were observed for any change in appearance or colour for the period of 4 weeks. There was no change in appearance in ethosomal formulation throughout the period of study. The stability of drug was further confirmed by spectral data and there was no change observed.

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