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FORMULATION AND *IN-VITRO* EVALUATION OF GEL CONTAINING ETHOSOMES ENTRAPPED WITH ETODOLAC

Pavan Kumar Chintala* and J. Padmapreetha

Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India

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Correspondence to Author:

Pavan Kumar Chintala

Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India

E-mail: pavanchowdary.chintala@gmail.com

ABSTRACT: Etodolac is an indole acetic acid derivative having half-life of 4 to 7 h and used for the treatment Rheumatoid arthritis. The oral use of Etodolac is not much recommended as it has many systemic side effects. The entrapment of drug in a vesicle has shown improved delivery of drug at the targeted site and has also reduced the dose and thus, has shown better patient compliance. Ethosomes are lipid vesicular carriers containing ethanol which provides better penetration of drug into the skin. Ethosomes of Etodolac were prepared by hot method. The composition includes phospholipid, ethanol, propylene glycol and distilled water. Liposomes of Etodolac were also prepared by thin film hydration technique. Selected formulations were subjected to sonication for reducing the vesicle size. FT-IR study confirmed the purity of drug and revealed no interaction between the drug and excipients. Ethosomes and liposomes were characterized for vesicle shape, vesicle size, entrapment efficiency percentage, *in vitro* drug diffusion. %CDR after 8 h for ethosomal, liposomal are 76.55 ± 0.70%, 65.61 ± 0.68% and respectively. Ethosomal formulation (F8) was found stable at 4 ± 2°C and at room temperature during the storage of 45 days. Efficient delivery of drug to deep skin strata from ethosomal drug application found to be highly beneficial in localizing the drug to desired site in the skin and reduced the side effects associated with conventional treatments.

INTRODUCTION: The skin covers a total surface area of approximately 1.8m^2 and provides the contact between the human body and the external environment.

Dermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases and other inflammatory conditions.

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This has the advantage that high concentrations of drugs can be localized at the site of action, reducing the systemic side effects. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs. The structure of stratum corneum is often compared with a brick wall, with the corneocytes as the bricks surrounded by the mortar of the intercellular lipid lamellae.

Many techniques have been aimed to disrupt and weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin. One of the most controversial methods is the use of vehicle formulations as skin delivery systems [1].

Ethosomes are novel carrier system used for delivery of drugs having low penetration through the biological membrane mainly skin. Ethosomes are the slight modification of well-established drug carrier liposome. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water.[2] Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers to microns (μ) [3].

Etodolac belongs to a class of drugs called nonsteroidal anti-inflammatory drugs (NSAIDs) [4]. These drugs are used for the management of mild to moderate pain, fever, and inflammation. They work by reducing the levels of prostaglandins, which are chemicals that are responsible for pain and the fever and tenderness that occur with inflammation [5]. Etodolac blocks the enzyme that makes prostaglandins (cyclooxygenase), resulting in lower concentrations of prostaglandins. As a consequence, inflammation, pain and fever are reduced.

MATERIALS AND METHODS:

Materials: Etodolac was obtained as a gift sample from Rachem Pharmaceuticals Ltd., Hyderabad. Soya Phosphotidyl choline From Himedia mumbai. Ethanol and Propylene glycol from SD Fine chemicals Mumbai. All other chemicals and reagent were of analytical grade.

Methods:

Preparation of Ethosomes:

Hot Method: Ethosomes were prepared by hot method. In this specified amount of phospholipid was dissolved in water and kept under magnetic stirrer for 30 min. The lipid mixture was heated at 40^0c on magnetic stirrer latter specified amount of Etodolac was added to ethanol to this propylene glycol was added and it was kept under magnetic stirrer latter lipid solution was added drop by drop and it was kept under magnetic stirrer for 1hr. The solution was kept at 40^oC for 1hr later it was kept for sonication using ultra sonicator for 15 min to reduce the particle size

Preparation of Liposomes [6]:

1. **Lipid Film Hydration Method:** Phospholipid and cholesterol were dissolved in little quantity of chloroform in a round bottom flask. The solvent was allowed to evaporate using vacuum evaporator to form a thin film of lipid on the wall of flask. Lipid film was then hydrated with the solution of drug in a phosphate buffer of pH 6.8 by magnetic stirrer for 1 h at R.T.

Preparation of Gel Base [7]: 1 % w/w gel base was prepared by dispersing Carbopol 934 in distilled water containing 0.2% methyl paraben and 0.02% propyl paraben, using magnetic stirrer. Here, Carbopol 934 was used as gelling agent and methyl paraben and propyl paraben were used as preservatives.

Preparation of Ethosomal Gel [8]: The Ethosomal sediments were collected and incorporated into the prepared gel base to get ethosomal gel.

Evaluation of Ethosomes of Etodolac:

1. **Entrapment efficiency [9]:** 10 ml of ethosomal solution was taken in Centrifuge tube. Ethosomal formulation was subjected to 5000 rpm for 1 hr. Using laboratory centrifuge the unentrapped drug concentration was determined spectrophotometrically at 274 nm. The drug entrapment percentage was calculated using the given equation.

Entrapment efficiency % = A_2-A_1/A_2×100

Where, A_1= Amount of Etodolac in sediment, A_2= Total amount of Etodolac added.

2. **Vesicle size:** The vesicle size of the best formulation was determined by using Dynamic light scattering.

3. **Vesicle shape:** The vesicle shape of best formulation was determined by using scanning electron microscopy.

4. **Optical microscopy observation [10]:** The ethosomal dispersion was spread on the glass slide using a glass rod. Formation of

multilamellar vesicles was confirmed by examining the ethosomal suspension under an optical microscope with the magnification power of 100 x.

5. **pH studies:** The pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter**.**

6. **Extrudability study** [14]: In the present study, the method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. The extrudability was than calculated by using the following formula:

Extrudability = Applied weight to extrude gel from tube (in gm) / Area (in cm2)

7. **Spreadability Coefficient studies** [13]**:** The Spreadability of the gel formulations was determined 48h after preparation, by measuring the spreading diameter of 10 g of the gel between two glass plates after 1min. The mass of the upper plate was standardized at 20 g, placing slides one above the other and count time taken for 2nd slide to slip out from other slide

Spreadability coefficient= ML/T

M=mass, L=length, T=time

8. ***In vitro*** **diffusion study** [11]**:** *In vitro* diffusion study was carried out in a Franz diffusion cell using cellophane membrane. The cellophane membrane was mounted on the Franz diffusion cell. Formulation was applied through donor compartment on the dialysis membrane. Reservoir compartment was filled with 25 ml phosphate buffer of pH 6.8 The study was carried out at 37 ± 1°C and at a speed of 100 rpm for 8 h. Samples were withdrawn from reservoir compartment at 1 h interval and absorbance was measured spectrophotometrically at 274 nm. Each time the reservoir compartment was replenished with the same quantity of 6.8 pH phosphate buffer.

9. **Stability studies** [12]**:** Stability testing of drug products begins as a part of drug discovery and ends with the commercial product. To assess the drug and formulation stability, stability studies were done. The stability studies were carried out for the most satisfactory formulation. The most satisfactory formulation was sealed in a container and kept at 4 ± 2°C and at R.T. for 45 days. At the end 45 days, the sample was analyzed for the entrapment efficiency percentage, and *in vitro* skin diffusion study.

RESULTS AND DISCUSSION: The present study was carried out to develop the Ethosomes of Etodolac by hot method. Hence, it was necessary to find suitable excipients with good compatibility. Ten formulations of Etodolac were prepared with different concentrations of soya phosphatidyl choline and Ethanol. Phospholipid was used as vesicle forming agent. Ethanol is used as penetration enhancer. Propylene glycol is used as viscosity forming agent. Distilled water is used as vehicle (**table 1**).

TABLE 1: FORMULATION CHART OF ETHOSOMES &LIPOSOMES CONTAINING ETODOLAC

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10*L
Etodolac (mg)	300	300	300	300	300	300	300	300	300	300
Phospholipid (mg)	300	300	300	600	600	600	900	900	900	900
Ethanol (ml)	6	9	12	6	9	12	6	9	12	-
Propylene glycol (ml)	3	3	3	3	3	3	3	3	3	-
Cholesterol (mg)	-	-	-	-	-	-	-	-	-	300
Chloroform (ml)	-	-	-	-	-	-	-	-	-	25
Dist. Water	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto 30ml	Upto3 0ml	25 ml

*L=Liposomes

Pre Formulation Study:

1. **Melting Point:** Melting point of Etodolac was found to be 146°C and which was found to be in the range of 144- 150°C.

2. **Solubility:** Etodolac is freely soluble in Ethanol. Sparingly soluble in 6.8 pH phosphate buffer and insoluble in water

Drug Polymer Compatibility:

1. **FTIR studies:** The drug excipient compatibility study was done by using SHIMADZU FT/IR spectrometer. The IR spectra for pure drug, and drug-excipient mixture were shown in **figures 7-9**. The spectrum of the pure drug and excipients suggests that there were no interaction between drug and excipients and were compatible with each other.

2. **Vesicle size:** The vesicle size was determined by Dynamic light scattering .It was found to be 242.9nm it was shown in **figure 11**.

3. **Vesicle shape:** Vesicle shape was determined by Scanning Electron Microscopy. It was shown in **figure 12.**

4. **Entrapment efficiency:** The entrapment efficiency was found to be higher in F8 formulation with 80.5 %. F10 formulation of liposomes showed entrapment efficiency of 69.33% which is lesser than liposomes. F8 formulation is selected as best formulation for further studies. it was shown in **figure 13**. The values are shown in **table 2**.

TABLE 2: DRUG ENTRAPMENT EFFICACY RESULTS OF ETHOSOMES & LIPOSOMES CONTAINING ETODOLAC

Formulation	% Drug Entrapped
F1	40.83
F2	50.16
F3	44.67
F4	57.71
F5	64.83
F6	61.16
F7	71.5
F8	80.5
F9	74.83
F10 L	69.33

5. ***In-vitro* Drug diffusion studies:** *In-vitro* drug release was observed more in F8 formulation with 76.55% and drug release in liposomes was found to be 65.61% decrease in drug release when compared to ethosomes was due to absence of Ethanol. The values are shown in **table 3**.

TABLE 3: IN-VITRO DRUG RELEASE

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10 *L
1	3.45±0.12	5.43±0.21	4.52±0.24	14.54±0.15	20.45±0.16	16.85±0.2	24.51±0.09	32.51±0.21	28.97±0.41	25.02±0.17
2	7.62±0.23	11.18±0.22	7.16±0.29	20.68±0.44	26.08±0.19	19.72±0.21	28.10±0.51	39.69±0.25	37.04±0.59	27.21±0.30
3	10.63±0.25	16.42±0.67	12.17±0.37	28.57±0.13	29.88±0.25	27.76±0.24	33.90±0.56	44.71±0.27	41.94±0.66	34.85±0.33
4	16.82±0.13	24.53±0.25	18.59±0.25	33.92±0.45	37.51±0.55	33.10±0.22	39.93±0.55	52.17±0.31	49.36±0.70	40.95±0.13
5	20.37±0.15	33.75±0.29	23.89±0.41	38.88±0.23	42.13±0.29	40.94±0.2	45.43±0.57	56.90±0.35	54.07±0.82	46.75±0.17
6	28.718±1.52	38.64±0.32	29.86±0.33	43.91±0.22	48.52±0.28	46.23±0.26	49.44±0.52	64.10±0.12	61.53±0.95	50.92±0.18
7	34.35±1.42	43.16±0.29	36.53±1.08	48.31±0.25	55.03±0.22	51.33±0.25	57.75±0.09	71.17±0.16	66.80±1.46	59.17±0.29
8	37.59±0.68	47.73±0.77	41.49±0.89	53.40±0.94	61.45±0.95	57.46±0.92	67.44±0.88	76.55±0.70	72.50±0.72	65.61±0.68

6. **FTIR of Ethosomes:** The FTIR report of best formulation of Ethosomes showed same characteristic bands as pure drug this confirm that drug Etodolac was incorporated in ethosomes. It is shown in **figure 10**.

7. **pH studies:** The pH of the best formulation was found to be 5.5 which was found to be in the acidic range. The gels that are applied to skin should be acidic in nature because the skin pH is acidic in nature.

8. **Spreadability coefficient studies:** The Spreadability coefficient of the best formulation was found to be 7.2gm cm/sec.

9. **Extrudability studies:** The Extrudability of the best formulation was found to be 1.12gm/cm².

10. **Release kinetics:** The release kinetics of ethosomes of Etodolac showed that the drug follows higuchi release and it follows non fickian release. There is no significance difference observed in other values. The values are shown in **table 4**. It was shown in **figures 4 & 5**.

TABLE 4: KINETIC MODELING DATA

Formulation	Zero order	First order	Higuchi model	Korsemeyer-Peppas	
	r^2	r^2	r^2	slope	r^2
F1	0.957	0.978	0.994	0.533	0.965
F2	0.966	0.986	0.992	0.633	0.996
F3	0.990	0.899	0.978	1.255	0.984
F4	0.992	0.859	0.954	1.539	0.978
F5	0.986	0.861	0.923	1.170	0.993
F6	0.899	0.993	0.975	0.442	0.982
F7	0.909	0.989	0.996	0.415	0.970
F8	0.939	0.970	0.987	0.481	0.938
F9	0.981	0.969	0.924	0.673	0.986

11. **Stability studies:** There was no significant change observed in entrapment efficiency and *in-vitro drug* release studies for the best formulation after 45 days .the values are shown in **table 5 & 6**. It was shown in **figures 6 & 17.**

TABLE 5: ENTRAPMENT EFFICIENCY % AFTER STABILITY STUDIES

Formulation code	Entrapment efficiency % after stability studies	
	At 4 ± 2°C	At R.T.
F8	79.16	76.33

TABLE 6: COMPARATIVE DIFFUSION PROFILE AFTER STABILITY STUDIES

TIME (h)	% CDR	
	At 4 ± 2°C	At R.T.
	F8	F8
1	22.15±0.63	20.15±0.63
2	28.75±0.57	26.94±0.74
3	38.81±0.67	35.81±0.63
4	46.45±0.62	43.71±0.68
5	52.62±0.47	49.41±0.58
6	61.13±0.51	57.81±0.68
7	65.72±0.61	63.72±0.69
8	72.69±1.07	70.45±1.20

SUMMARY: A novel ethosomal system has been developed for transdermal delivery. Etodolac is generally given by oral route however it has several adverse effects like gastric ulceration bleeding and first pass metabolism etc. To overcome these limitations, alternative transdermal route has been selected. Its permeability is increased by using ethanol in the formulation in the present work ethosomes of Etodolac were prepared by hot method and evaluated. Liposomes were prepared by thin film lipid hydration method. The prepared ethosomes were characterised for entrapment efficiency, vesicle size, vesicle shape, compatibility studies and stability studies. Ethosomal gel was evaluated for *in vitro drug* release, pH, and Spreadability studies. Thus, the prepared ethosomes was proved to be potential candidate for Transdermal delivery system.

CONCLUSION: From the experimental results it can be conclude that. From the IR spectra it can be revealed that there was no interaction between drug and excipients hence they are compatible. % Entrapment efficiency and *in vitro* drug release was higher for F8 formulation. Particle size was analysed by DLS and found that to be 242 nm Vesicle shape was analysed by SEM. From all the parameters it can be conclude that ethosomes are better than liposomes.

As the concentration of ethanol increases % drug release and %entrapment efficiency decreased. As the concentration of Phospholipid increases %drug release and %entrapment efficiency increased. Optimized formulation followed Higuchi plot and non-Fickian release. It can be conclude that ethosomes are potential candidate for Transdermal drug delivery.

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