



## Formulation and Evaluation of Ethosome (Mefenamic Acid) Using Hot Method

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### ABSTRACT

*Ethosomes are amphiphilic novel lipid vesicular systems that have shown their potential in improving the bioavailability of poorly water soluble as well as poorly lipophilic drugs. Mefenamic acid is anthranilic acid derivative agent and belongs to Biopharmaceutical classification system class II drugs that show poor water solubility as well as poor lipophilic character. Oral use of Mefenamic acid contraindicated resulting from sever side effect, thus topical administration is recommended. The present research work Ethosomal vesicles were successfully developed through hot method under stirring and sonicated and evaluated DSC thermograms, Particle size distribution, Vesicles shape, in vitro Permeation study and Stability study. The present study demonstrates that the ethosome vesicle found to be Particle size distribution was found good. Stability study was found 97.85%, 98.69% and 98.43% as respectively. In vitro drug release 94.509%, 95.957% and 88.068% respectively as per formulation (MET1, MET2, and MET3). The entrapment of drug in vesicles found to be 57.98%, 67.26% and 33.19% respectively, solubility and availability of drug at the site.*

**Keywords:** Vesicular system, Ethosomes, Mefenamic acid

### INTRODUCTION

Ethosomal drug delivery system is non-invasive and delivers the drug to the deep skin layers to systemic circulation. Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol and water having a size range from tens of nanometres to microns. Size of ethosomes depends upon the method of preparation and application of techniques like sonication. These “soft vesicles” represents novel vesicular carrier for enhanced delivery through skin. The soft, malleable vesicles tailored for enhanced delivery of active agents [1,2]. High concentration of alcohol (20-45%) in the formulation provides soft, flexible characteristics and stability to the vesicles and it also disrupts lipid bilayer structure of the skin results in an increase in the membrane permeability [3]. Ethosomes causes disturbance of skin lipid bilayer organization when they incorporate in to a vesicle membrane and it enhances the vesicles ability to penetrate the stratum corneum. The synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulation have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayer [4]. These vesicular systems were found to be highly ancient carriers for the delivery of molecules with various lipophilicities into and through the skin, in *in-vitro* and *in-vivo*, in animal and clinical studies The use of lipid vesicles, such as liposomes, as safe vehicles for topical drug-delivery systems has attracted increasing attention due to their effectiveness in entrapping drugs and delivering them to the skin [5]. Transdermal route is the most successful innovative research area in drug delivery. Ethanol in the ethosomes increases the cell membrane lipid fluidity, which results in increased skin Penetrability of ethosomes [6].

## MATERIALS AND METHODS

Mefenamic acid was gift sample from Hellios pharmaceuticals Baddi (H.P.), Soya phosphatidyl choline was purchased from Sigma Aldrich (Mumbai). Propylene Glycol, Ethanol from Nice chemicals. All other materials used in the study were of analytical grade.

### Preparation of Ethosome by Hot Method

According to this method, phospholipid was dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. Ethanol, propylene glycol and drug was mixed in a separate vessel and heated upto 40°C. Organic phase was added to aqueous phase and stirred for 5 min. The vesicle size of ethosomal formulation was decreased to desire extent using sonication. Finally, the formulation was properly stored [7].

Table 1: Preparation formula of Mefenamic ethosomes

Formulation Code	Phospholipid (%w/w)	Ethanol (%v/v)	Propylene Glycol (%v/v)	Drug (%w/w)
MET <sub>1</sub>	1	20	10	1
MET <sub>2</sub>	2	30	10	1
MET <sub>3</sub>	3	40	10	1

## EVALUATION OF ETHOSOMES

### Vesicle Size and Zeta Potential

Particle size of vesicle can be determined by dynamic light scattering. The charge of the ethosomal vesicle is an important parameter than can influence both vesicular properties such as stability as well as skin- vesicle interactions and its zeta potential can also be determined using a computerized inspection system. The size of the vesicles can be characterized by light microscopy with an eye piece micrometer which is calibrated with a stage micrometer [7].

### Optical Microscopy Observation and Vesicle Shape

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. The vesicle shape of best formulation was determined by using scanning electron microscopy [8].

### Differential Scanning Colorimetry (DSC)

Differential scanning calorimetry (DSC) is used to determine the transition temperature of vesicular lipid system. Lipid bilayer exhibit various phase transitions that are studied for their roles in triggered drug release. Lipid bilayer can exist in a low-temperature solid ordered phase and above a certain temperature in a fluid-disordered phase. The temperature of this phase transition can be tailored by selecting the proper lipids [9].

### Entrapment Efficiency

Entrapment efficiencies of ethosomes formulations were carried out by centrifugation method. The ethosome was centrifuged at 8000 rpm for 10 min at 4°C. Then the solid mass was separated from the supernatant and the suitable dilutions were prepared with PBS (pH 7.4). The drug concentration was assayed by U.V spectrophotometer method at 279 nm. The percentage of drug entrapment was calculated by the following equation [10].

$$\text{Entrapment efficiency} = (T-C) \times 100/T$$

Where: T is total amount of drug that is detected both in the supernatant layer and resident layer.

C is the amount of drug detected only in the supernatant.

### In vitro Drug Permeation Study

The *in vitro* permeation study was carried out by using modified Franz diffusion cell with egg membrane. The study was performed with phosphate buffer saline (pH 7.4). The formulation was placed (equivalent to 2.5 mg of drug) on the upper side of skin in donor compartment. The temperature of the assembly was maintained at 37 ± 2°. Samples were withdrawn after every hour from the receptor media through the sampling tube and at the same time, same

amount of fresh receptor media was added to make sink condition. Withdrawn samples were analyzed for aceclofenac constant using UV spectrophotometer [11].

### Stability Study

Stability study was carried out for Mefenamic ethosomal preparation at two different temperature i.e. refrigeration temperature ( $4 \pm 2^\circ\text{C}$ ) and at room temperature ( $27 \pm 2^\circ\text{C}$ ) for 8 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any soil of interaction between the ethosomal preparation and glass of container, which may affect the observations [12].

## RESULTS AND DISCUSSION

### Zeta Potential

Microscopic analysis was performed under different magnification to visualize the vesicular structure lamellarity and to determine the size of ethosome preparation. The effect of phospholipids concentration on the size and distribution of ethosomes vesicles was investigated by using Malvern Zetasizer (Figures 1-3).

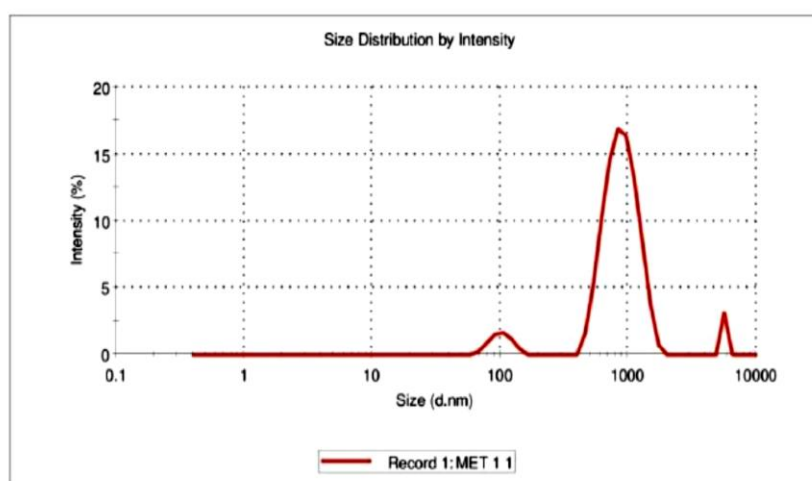


Figure 1: Zeta potential distribution plot of MET<sub>1</sub> formulation

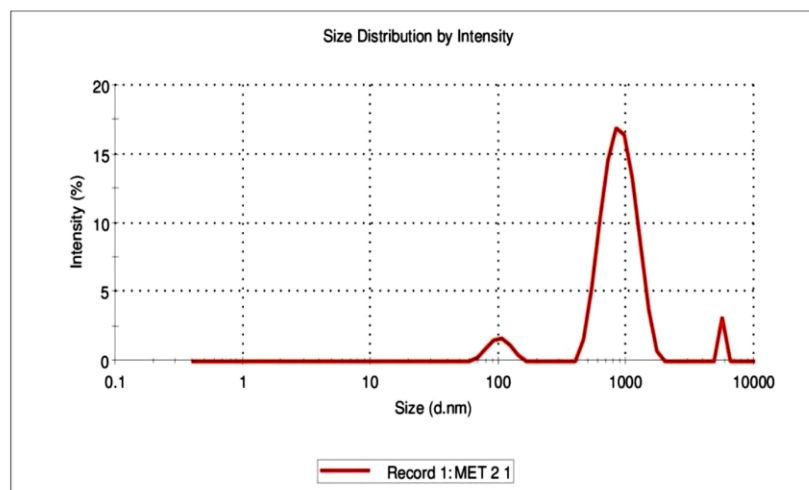
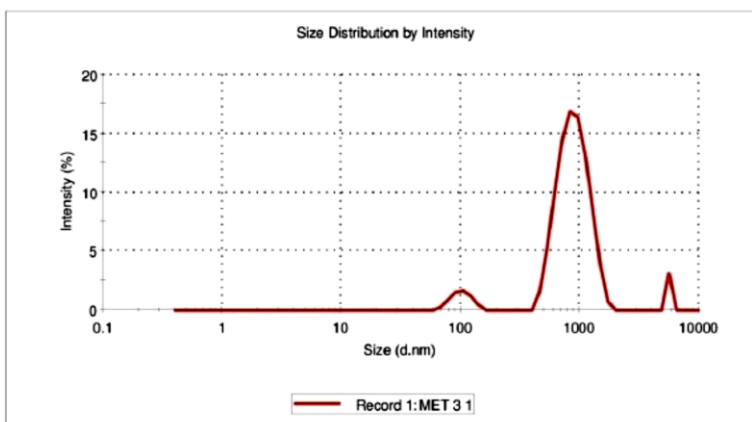


Figure 2: Zeta potential distribution plot of MET<sub>2</sub> formulation



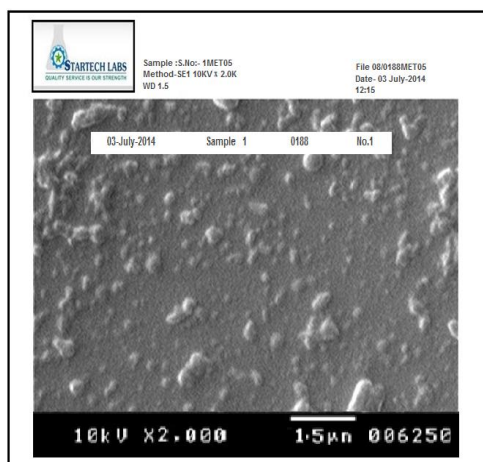
**Figure 3:** Zeta potential distribution plot of MET<sub>3</sub> formulation.

**Visualization of Vesicals by Phase Contrast Microscopy and Scanning Electron Microscopy (SEM)**

The vesicles were uniform in size and appeared to be multilayered. Visual observation of Ethosomes confirmed the multi-lamellar vesicular structures. Photomicrographs were taken using video camera connected to microscope with the help of computer installed with special software proved the multi-lamellar vesicular structure and spherical shape of Ethosomes of Mefenamic acid. Phospholipid vesicle can be confirmed by further analysis by scanning electron microscopy (SEM) (Figure 4 and Figure 5).



**Figure 4:** Photomicroscopy of Ethosome formulation



**Figure 5:** SEM of Ethosome formulation

### Size and Shape Analysis

The Ethosomal dispersion was spread on glass slide. Formation of multilamellar vesicles was confirmed by examining the Ethosomal dispersion under an optical microscope with magnification power of 100X. The examination of prepared formulations revealed the predominance of spherical shaped vesicles. The vesicles were uniform in size and appeared to be multilayered. Visual observation of Ethosomes confirmed the multi-lamellar vesicular structures. Photomicrographs were taken using video camera connected to microscope with the help of computer installed with special software proved the multi-lamellar vesicular structure and spherical shape of Ethosomes of Mefenamic acid. The ethosomal formulation (MET<sub>1</sub>) prepared with 20% (w/v) ethanol and 1% (w/v) phospholipids show an average vesicle size of 1.9625  $\mu\text{m}$ . When concentration of ethanol was increased the size of the vesicles decreased, the smallest vesicles were observed in preparation containing 30% ethanol which was 1.73125  $\mu\text{m}$  (MET<sub>2</sub>) and largest in preparation containing 40% ethanol 1.98125  $\mu\text{m}$  (MET<sub>3</sub>) were observed during study (Table 2 and Table 3) (Figure 6 and Figure 7).

Table 2: Size distribution of Mefenamic Ethosome formulation

Size range			Formulations					
			MET <sub>1</sub>		MET <sub>2</sub>		MET <sub>3</sub>	
Eye Piece Micrometer Division	Calibrated Stage Micrometer ( $\mu\text{m}$ )	Average Size (d)	No. of Vesicles (n)*	% Vesicles	No. of Vesicles	% Vesicles	No. of Vesicles (n)*	% Vesicles
0-1	0-1.25	0.625	80	40	92	46	78	39
01-Feb	1.25-2.5	1.875	60	30	60	30	58	29
02-Mar	2.5-3.75	3.125	40	20	32	16	44	22
03-Apr	3.75 – 5	4.375	10	5	12	6	12	6
04-May	5-6.25	5.625	6	3	3	1.5	5	2.5
05-Jun	6.25-7.5	6.875	4	2	1	0.5	3	1.5

Table 3: Average size distribution of mefenamic ethosomes formulation

S. No.	Formulation	Average Size ( $\mu\text{m}$ )
1	MET <sub>1</sub>	1.9625
2	MET <sub>2</sub>	1.73125
3	MET <sub>3</sub>	1.98125

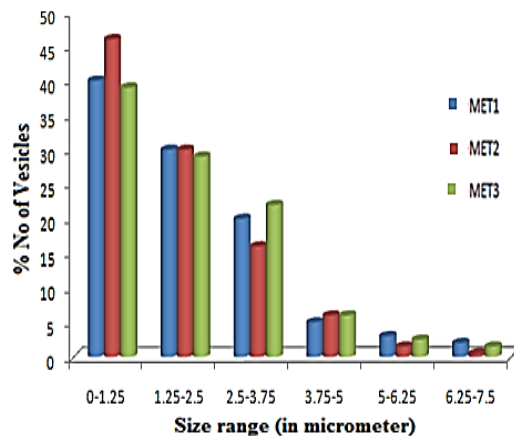


Figure 6: Size distribution of ethosomes formulation

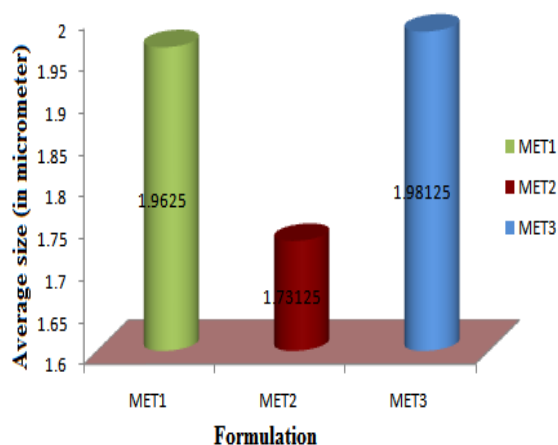


Figure 7: Average Size distribution Ethosome formulation

### Incompatibility Study of Formulation

The Incompatibility Study of Formulation done by DSC. DSC thermograms of pure drug and corresponding drug carrier system are depicted in the DSC curve of Mefenamic Acid shows a sharp endothermic peak ( $T_{\text{peak}} = 232.38^{\circ}\text{C}$ ) corresponding to its melting, indicating its crystalline nature. Phospholipid showed a sharp endotherm at  $13.91^{\circ}\text{C}$ ,  $22.32^{\circ}\text{C}$ , corresponding to its transition temperature. The mixer showed a sharp endotherm at  $204.03^{\circ}\text{C}$  and  $218.29^{\circ}\text{C}$  corresponding to its melting point/transition temperature. DSC thermogram of Mefenamic loaded thermogram was observed at  $208.38^{\circ}\text{C}$  and  $235.68^{\circ}\text{C}$ . This signifies that incorporated Mefenamic has interacted well with phospholipid. The absence of Mefenamic melting thermogram also proves the enhanced entrapment of drug within the vesicles. There was no appreciable change in the melting endotherms of the physical mixture (mefenamic+phospholipid) compared to pure drug (Figure 8).

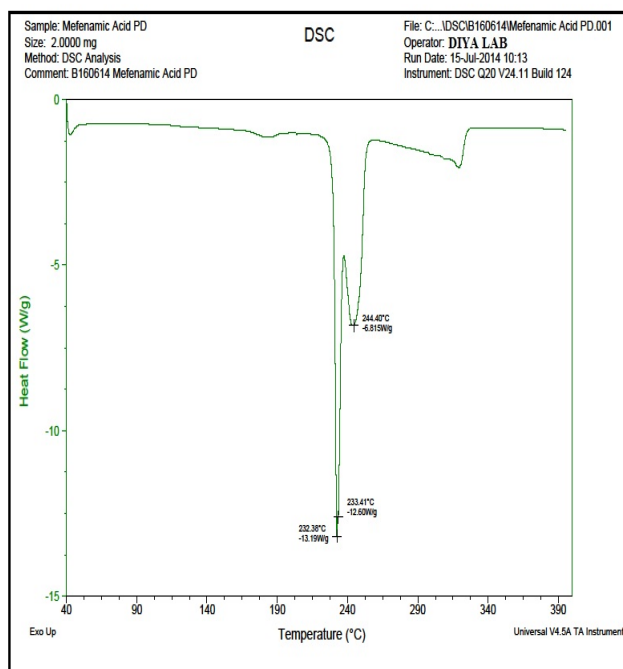


Figure 8a: DSC thermograms of mefenamic acid Mefenamic ethosome

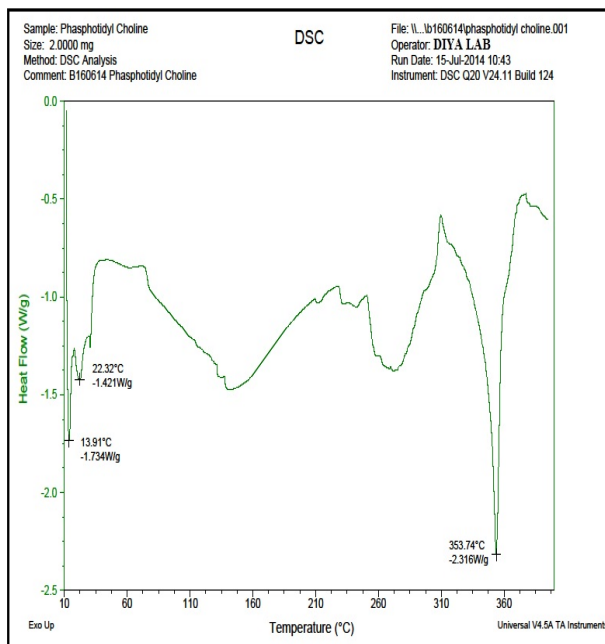


Figure 8b: DSC thermograms of phosphatidyl coline

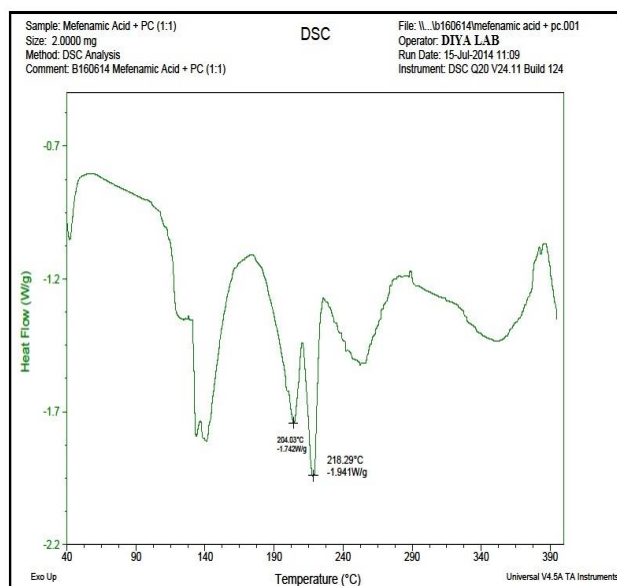


Figure 8c: DSC thermograms of Mefenamic +phosphatidyl coline

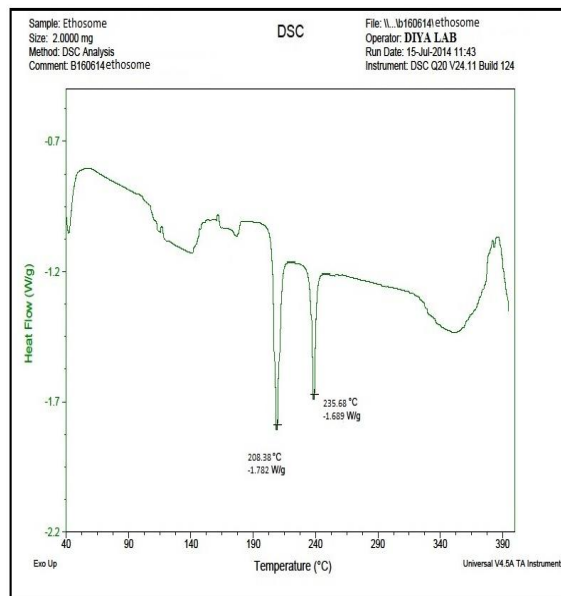


Figure 8c: DSC thermograms of Mefenamic Ethosome

### Entrapment Efficiency

The presence of bilayer vesicles was confirmed in the Ethosomal system, the ability of vesicles for entrapment of drug was investigated by ultracentrifugation. Ultracentrifugation was the method used to separate the ethosomal vesicles containing drug and untrapped or free drug, to find out the entrapment efficiency. The maximum entrapment efficiency of ethosomal vesicles as determined by ultracentrifugation was 67.26% for ethosomal formulation containing 30% ethanol (MET<sub>2</sub>) which was almost double to the formulation containing 40% ethanol (MET<sub>3</sub>). As the ethanol concentration increased from 20% to 30% w/w, there was increase in the entrapment efficiency and with further increase in the ethanol concentration (>30% w/w) the vesicle membrane becomes more permeable that lead to decrease in the entrapment efficiency. Results of entrapment efficiency also suggest that 2% phospholipid is optimal concentration for entrapment efficiency and hence increased or decreased in concentration of phospholipid reduces the entrapment efficiency of vesicles (Figure 9) (Table 4).

Table 4: Drug entrapment efficiency of ethosomes.

Sample		Absorbance	Conc (µg/ml)	Amount of Drug CxDF (µg)	Entrapped Drug E=T-U (µg)	% Drug Entrapped =E/T × 100
MET <sub>1</sub>	Total drug (T)	0.5912	9.747	1949.4		
	Free drug (U)	0.2496	4.095	819	1130.4	57.98
MET <sub>2</sub>	Total drug (T)	0.5783	9.533	1906.6		
	Free drug (U)	0.1904	3.122	624.4	1282.3	67.26
MET <sub>3</sub>	Total drug (T)	0.6014	9.9454	1989.08		
	Free drug (U)	0.4035	6.64	1328.8	660.28	33.19



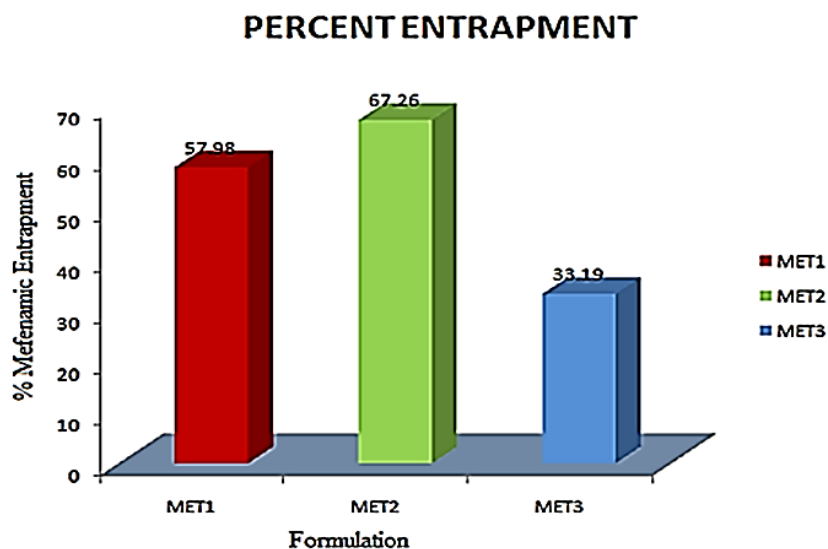


Figure 9: Entrapment efficiency of different ethosomes vesicles

### ***In vitro* Skin Permeation Studies**

*In vitro* skin permeation study or *in vitro* skin diffusion study have been extensively studied using Franz diffusion cell and epidermal surface of rat skin. The objectives in the developments of *in vitro* diffusion tests are to show the release rates and extent of drug release from dosage form. Skin permeation study was carried out for 96 hours duration; all results were shown on table and represented graphically.

For the formulation MET2 with (1:2) drug-lipid concentration, the percentage drug release after one hour was only  $0.578 \pm 0.08\%$ , which was improved to  $15.960 \pm 0.12\%$  after 4 hours of diffusion. The percentage drug release from MET2 was almost doubled to  $30.591 \pm 0.17\%$  after 12 hours of study. After 24 hours of diffusion, the percentage drug release was  $39.243 \pm 0.18\%$ . 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  $54.646 \pm 0.16\%$  and  $70.346 \pm 0.19\%$  respectively for MET<sub>2</sub>. At the end of diffusion study after 96 hours, the percentage drug release from MET2 was  $95.957 \pm 0.05\%$  and 4.043% was remaining within the MET2 (Figure 10) (Table 5).

Table 5: *In vitro* drug release data for formulation MET<sub>1</sub>, MET<sub>2</sub>, MET<sub>3</sub>

Time in Hours	Dilution Factor	%Drug Released MET1	%Drug Released MET2	%Drug Released MET3
0	10	0	0	0
1	10	0	0.578	12.23
2	10	1.07	6.829	22.765
4	10	7.293	15.96	33.096
6	10	13.739	20.237	43.11
12	10	21.222	30.591	52.116
24	10	32.857	39.243	59.065
36	10	43.187	45.515	66.712
48	10	53.711	54.646	74.592
60	10	70.001	70.346	84.465
72	10	85.452	83.439	87.814

84	10	93.335	90.733	88.037
96	10	94.509	95.957	88.068

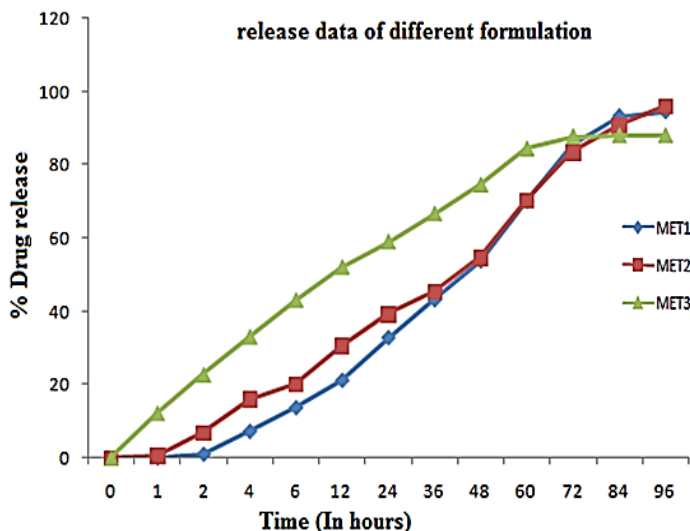


Figure 10: *In vitro* cumulative drug release of ethosomes formulation (MET<sub>1</sub>, MET<sub>2</sub>, MET<sub>3</sub>)

**Stability Study of Ethosome**

The accelerated stability studies were carried out in accordance with the ICH guidelines. The ability of vesicles to retain the drug was assessed by keeping the Ethosome suspension at different temperature. Optimized Ethosomes formulations were selected for stability studies of vesicles. Since the stability of drug and stability of vesicles are the major determinant for the stability of formulation, studies were carried to evaluate total drug content (Table 6) at room temperature ( $27 \pm 2^\circ\text{C}$ ) and refrigeration temperature ( $4 \pm 2^\circ\text{C}$ ). Stability study could not be carried out at higher temperature because phospholipid was used as on the component for ethosomes and gets deteriorated at higher temperature. Loss in the percentage of drug content was not more than 2%. Highest drug loss was observed at room temperature after 8 weeks as compared to refrigeration temperature. Results also showed that there was no significant change. The drug content in different formulations, indicating the stability of drug even after 8 weeks. Entrapment efficiency is the integral part of stability of vesicles, hence selected to show the stability of prepared formulation. According to the data (Table 7) indicate that formulations stored at refrigeration temperature were found to show higher entrapment efficiency (Figure 11 and Figure 12) and (Table 6 and Table 7).

Table 6: Data of stability studies for drug content at two different temperatures

Formulation	Drug Content in%									
	Initial		After 2 Weeks		After 4 Weeks		After 6 Weeks		After 8 Weeks	
	4 ± 2°C	27 ± 2°C	4 ± 2°C	27 ± 2°C	4 ± 2°C	27 ± 2°C	4 ± 2°C	27 ± 2°C	4 ± 2°C	27 ± 2°C
MET <sub>1</sub>	100	100	99.85	99.8	99.66	98.83	99.24	98.01	98.74	97.85
MET <sub>2</sub>	100	100	99.74	99.52	99.56	99.21	99.43	99	99.17	98.69
MET <sub>3</sub>	100	100	99.75	99.49	99.53	99.89	99.44	98.55	99.13	98.43

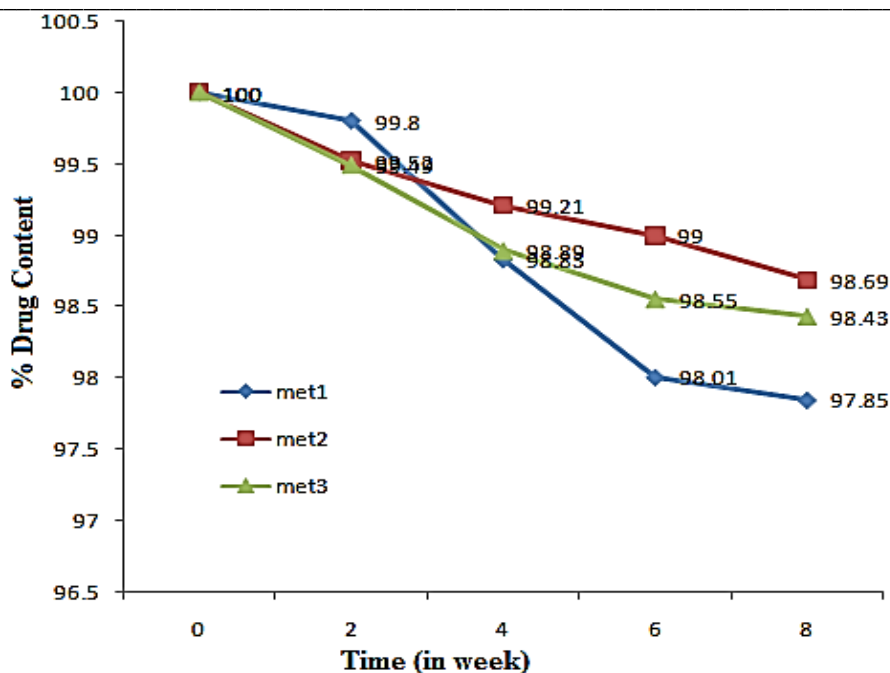


Figure 11: Loss of Percentage Drug content of the vesicles (MET<sub>1</sub>, MET<sub>2</sub>, MET<sub>3</sub>) during stability study

Table 7: Data of stability studies for entrapment efficiency at two different temperatures

Formulation	Entrapment study									
	Initial		After 2 Weeks		After 4 Weeks		After 6 Weeks		After 8 Weeks	
	4 ± 2 °C	27 ± 2 °C	4 ± 2 °C	27 ± 2 °C	4 ± 2 °C	27 ± 2 °C	4 ± 2 °C	27 ± 2 °C	4 ± 2 °C	27 ± 2 °C
MET <sub>1</sub>	58.78	58.78	58.17	57.31	57.91	55.29	57.72	54.11	57.43	52.24
MET <sub>2</sub>	71.48	71.48	71.13	70.56	70.58	68.39	70.3	66.03	69.71	64.17
MET <sub>3</sub>	43.01	43.01	42.56	42.03	42.21	40.67	41.87	39.55	41.22	38.35

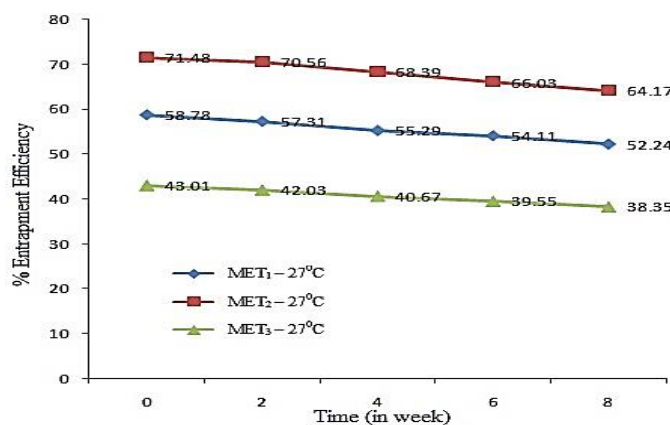


Figure 12: Loss of Percentage Drug entrapment efficiency of MET<sub>1</sub>, MET<sub>2</sub>, MET<sub>3</sub> during stability study.

### CONCLUSION

The ethosomal formulation of Mefenamic was prepared with the view to improve therapeutic response and reduce the possible adverse symptoms. Here ethosomes of Mefenamic acid were prepared using Hot process. Percentage

entrapment efficiency was optimized after studying the effect of various levels of ethanol in the formulation. Maximum entrapment efficiency was found to be 67.26% with 30% ethanol concentration. The prepared ethosomes were found to have good morphological properties and size distribution. From DSC thermograms of ethosomes it can be concluded that significant interaction occur between drug and lipid components of the vesicles that lead to higher entrapment efficiency. The percentage drug release from the Mefenamic Ethosomes was found to be 95.957% after 96 hours of *in vitro* studies. Stability studies showed maximum percent drug retention capabilities of ethosomes. These results conclusively demonstrated provide better relief from the disease and reduces the duration of therapy.

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