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FORMULATION AND EVALUATION OF CAPTOPRIL ETHOSOMES AS VESICULAR CARRIER

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ABSTRACT

The current investigation aims to evaluate the transdermal transport of vesicular carrier ethosome. Captorpil ethosomal carriers were prepared, optimized and characterized for vesicular size, entrapment efficiency and *In-vitro* drug release study. Ethosome formulation were prepared(F_1-F_5) by keeping drug, lecithin, propylene glycol concentration as constant as 0.05% w/w, 2% w/w and 10% w/w respectively and changing the concentration of ethanol by 20, 30, 40,50,60%w/w from F1 toF5. The effects of different concentration of ethanol were studied. The size of the vesicles were observed as decreased with increasing the ethanol concentration from 20-40% and shows no effect on the size at further increasing ethanol concentration to 50-60%. F3 ethosomes with 40%w/w ethanol were found to show highest captopril release (79.78 ±0.60). Vesicle size of F3 was found to be (92±9.0%) and zeta potential was (-17.6±2.30). In final phase of formulation development, F3 ethosomes were converted into gel (F3-G1, F3-G2, F3-G3) using three different carbopol concentrations (1.0, 1.5, 2.0%w/w). Captopril encapsulated in F3-G2 ethosomes in 1.5%gel was found to have shown maximum *In-vitro* drug release (85.21±1.23%) as compared to other carbopol concentrations and free drug gel (G4).

KEYWORDS

Ethosome, Ethanol, Propylene glycol, Soya lecithin and Rabbit skin.

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INTRODUCTION

The major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin. Transdermal delivery is important because it is a non-invasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration, gastric irritation and discomfort

associated with parenteral drug administration can be avoided. Flexible liposomes are common vectors in transdermal drug-delivery systems, with relatively good liquidity and deformability[1]. In recent years, ethosomes have become new liposome carriers with high deformability; high entrapment efficiency and a good transdermal permeation rate in the drug-delivery system, and are suitable for transdermal administration[2]. Compared with other liposomes, the physical and chemical properties of ethosomes make these more effective for drug delivery through the stratum corneum into the blood circulation, which is very important in the design of a transdermal drug-delivery system[3].

Captopril is an orally active inhibitor of angiotensin converting enzyme and it is widely used in the treatment of hypertension and congestive cardiac failure. The bioavailability of captopril is approximately 65 % have relatively short half-life of just 3 hours and requires frequent administration of dose 25 - 50 mg, 2-3 times daily. Hence, it is necessary to develop sustained release formulation to overcome this draw back.

MATERIAL AND METHODS

Materials

Captopril was a kind gift from Micro labs, Bangalore. Soya lecithin is obtained as a gift sample from Sonic Biochem Extractions Ltd., Indore. Carbopol® 940 was obtained from Rolex Chemical Industries, Mumbai. Ethanol absolute (99.9%) was obtained from Merck, Mumbai. Propylene glycol was obtained from Thomas Baker (Chemicals) Pvt. Ltd, Mumbai. Triethanolamine was obtained from s.d fine-chem limited, Mumbai. Water used for all experimental purposes was type-I (Millipore®). Animals (*rabbit*) and animal skins were obtained from animal house, All animal experiments were carried out as per approved protocols by recognized by pharmacy council of India and Govt. of Karnataka and AICTE (Affiliated to RGUHS, Bangalore).

Preparation of captopril loaded ethosomal vesicles

Ethosomal formulation was prepared according to the method reported by the Touitou et al[4]. Soya lecithin and captopril was dissolved in ethanol in a covered vessel at room temperature by vigorous stirring. Propylene glycol was added during stirring. This mixture was heated to 30°C in a water bath. The water was heated to 30°C in a separate vessel and was added to the mixture drop wise in the centre of the vessel, which was stirred for 5 min at 700 rpm in a covered vessel. Ethosomes were formed spontaneously by the process. Composition of different ethosomal formulations are given in Table No.1 and 2.

Characterization of vesicles:

Particle size analysis of ethosomes[5]

Particle size was measured using the MVtex SM-3JR optical microscope. Immediately after diluting and filtering the ethosomes with a 0.22 μm filter. The average particle size of ethosomes was calculated based on the measurements of 5 batches of ethosomes (Figure No.1).

Entrapment efficiency

The entrapment efficiency of the vesicles was determined by ultracentrifugation method. One ml of the formulation was centrifuged at 4ºC at 14,000 rpm for 1 hr. The supernatant containing the unentrapped drug was decanted. The vesicles were lysed using Triton-X 100 (0.1%v/v) and after further dilutions it was analyzed for drug content using UV Spectrophotometer at 219nm[6, 7]. The entrapment efficiency was expressed as percentage of total drug entrapped using the following formula[8].

$$\text{Percentage Entrapment} = \frac{C}{T} \times 100$$

Where, T = theoretical amount of drug that was added, and

C = amount of T drug detected after dissolving the vesicles.

Zeta potential determination[9]

Zeta potential of the vesicles was determined using Zetasizer (Nano-ZS, Malvern, U.K.).

The measurements were made in triplicate.

Turbidity measurement

Turbidity of all ethosomal vesicular suspensions was measured by SYSTRONICS Nephalometer. In this method, 500 NTU (Nephalometric Turbidity Units) range is set. Then zero reading is set with Millipore water. After this, formulation is transferred to glass cuvettes of capacity 50ml and placed in the holder inside the instrument. The method is repeated for each formulation and measurement of turbidity is displayed on the screen and expressed as NTU.

***In-vitro* permeation studies through rabbit skin**[10]

The *In-vitro* skin permeation of captopril from different formulations was studied using Franz glass diffusion cells. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm^2 and 10 ml respectively. The temperature was maintained at $32^0c \pm 1^0c$. The receptor compartment contained PBS (10ml of ph 7.4). Excised skin was mounted between the receptor compartment. Ethosomal formulation (1.0 ml) was applied to the epidermal surface of skin. Sample (0.5 ml) were withdrawn through the sampling port of the diffusion cell at 2-,4-,6-,8-,10-,12-,14-,16-,18-,20-,22-,and 24- hour time intervals and analyzed by ultra violet Spectrophotometry (UV) assay. An equal volume of fresh phosphate buffer maintained at $32^0c \pm 1^0c$ was replaced into the receptor compartment after each sampling. All investigations were performed after approved by the Institutional ethical committee and in accordance with the disciplinary principles and guidelines of the committee for the purpose of control and supervision of experiments on animals.

RESULTS AND DISCUSSION

Characterization of Vesicles

Vesicle size

The effect of ethanol concentration on the size distribution of ethosome vesicles was investigated and the investigation showed that the size of the vesicles decreased with increasing ethanol concentration from 20-40% (Table No.3 and Figure No.2).

This result indicates that at high ethanol concentration the membrane thickness is reduced considerably, probably due to the formation of a phase with interpreting hydrocarbon chain[11]. Further, ethanol causes a modification of the net charge of the system and confers it some degree of steric stabilization that may finally lead to a decrease in the mean particle size[12].

Entrapment efficiency

Drug entrapment within a vesicular carrier is one of the important features to evaluate the potential of the vesicular drug delivery system. For this reason, the entrapment efficiency of captopril within the ethosomal vesicles was determined for all formulations. Effect of ethosome composition i.e. ethanol concentration on captopril loading capacity was also investigated.

Different concentration of ethanol used for ethosomes preparation has a marked influence on the entrapment efficiency in ethosomes vesicles. The entrapment efficiency was found to vary with the varying concentration of ethanol. Entrapment efficiency was found to increase with ethanol concentration range i.e. 20-40% w/w. With the concentration of lecithin in range of 2%. The entrapment was found to be maximum in vesicles of F-2 (74.3±1.26) and minimum in F-4 (55.6±1.31) (Table No.3 and Figure No.3).

Zeta potential determination

Zeta potential is an important parameter that affects the aggregation of vesicles and depicts the physical stability of vesicular systems. Zeta potential of optimized formulation F-3 was found to high (-17.6±2.30) as shown in Table No.3. High zeta potential prevents the aggregation between vesicles and hence, enhances its physical stability. It has been investigated that high zeta potential in ethosomes increase the interbilayer distance owing to electrostatic repulsion[13].

Turbidity measurement

Turbidity measurement is an important parameter to study effect of ethanol concentration on lipid layer of ethosome vesicles. It was observed that turbidity measured in Nephelometric Turbidity Units (NTU) increased with increasing ethanol concentration. At low concentration range (20-40w/w), it increases the fluidity of lipid bilayers resulting in increased

turbidity of vesicle dispersion. At higher ethanol concentration (40-60%w/w) rapid decrease in turbidity of vesicle dispersion occurs. It is because after sublytic concentration, further increase in ethanol concentration solubilises the lipid bilyers that leads to decrease in turbidity of vesicle dispersion (Table No.3 and Figure No.4)[14].

***In-vitro* permeation studies through dialysis membrane**

The drug release profiles from all ethosomal dispersions showed rapid drug release during the initial phase from all formulations. However, during the following 24 hour a slow but prolonged release occurred whereas from formulation F3 79.78±0.60% of captopril was released.

Total percentage of drug release after 24h was found to be different among different formulations. With different ethanol concentration in ethosomes, the release was found to increase with initial increase in ethanol concentration (20-40%w/w) and then was found to decrease with ethanol concentration (>40%). This could be because of the disruption of the lipid bilayer when ethanol is present in high concentration.

***In-vitro* drug release studies of ethosomal gel**

Captopril ethosomal gels were prepared with different concentrations of carbopol ® 940. On comparing the release profile from various ethosomal gels it was evident that F3-G2 shows maximum release. Though there was not much difference in release between F3-G1 and F3-G2. But it was found consistency of F3-G1 was less as compared to F3-G2. Also it was found out that release from F3-G3 was much less as compared to other two probably due to the high viscosity attributed to high carbopol concentration. Now since, F3-G2 was found to have shown maximum release and good consistency, it was considered favorable for final development of the formulation (Table No.4 and Figure No.5).

A comparative study of the release profile of the optimized gel of captopril F3-G2 and gel containing free drug was found to show significant differences. F3-G2 had shown fast release instantaneously which gives an added advantage of F3-G2 over free drug gel (Table No.5). Thus, the rapid onset and maximum release of captopril from gel containing ethosomes as compared to free drug gel proved the effectiveness and efficiency of this vesicular carrier system in enhancing the transdermal delivery of captopril.

Table No.1: Formulation of ethosomes at different concentration of ethanol

S.No	Composition (% w/w) in 20ml					
	Formulation code	**Drug**	**Lecithin**	**Ethanol**	**Propylene glycol**	**Water**
1	F_1	0.05	2	20	10	Qs
2	F_2	0.05	2	30	10	Qs
3	F_3	0.05	2	40	10	Qs
4	F_4	0.05	2	50	10	Qs
5	F_5	0.05	2	60	10	Qs

Table No.2: Composition of various Captopril gel formulation (% w/w)

S.No	Gel formulation code	Captopril (in vesicles)	Carbopol (%w/w)	Triethanolamine (%w/w)	Phosphate buffer
1	F3-G1	0.05	1	0.5	Qs
2	F3-G2	0.05	1.5	0.5	Qs
3	F3-G3	0.05	2.0	0.5	Qs
4	*G-4	0.05	1.5	0.5	Qs

* G-4 Contains free drug

Table No.3: Comparative analysis of Ethosomal vesicles

S.No	Formulation	Vesicle size (nm) ± SD	Entrapment efficiency	Turbidity (NTU) ± SD	Zeta potential (mV) ± SD	% CDR ± SD
1	F1	174±6.2	69.5±0.35	212±6.3	-22.2±1.69	68.46±0.23
2	F2	102±8.5	74.3±1.26	269±3.5	-18.2±1.54	74.73 ±2.06
3	F3	92±9.0	66.8±0.32	294±0.2	-17.6±2.30	79.78 ±0.60
4	F4	152±1.3	55.6±1.31	315±8.0	-15.2±0.22	75.27 ±2.05
5	F5	143±1.6	57.9±0.92	184±3.4	-12.3±1.38	69.92±1.59

Table No.4: *In-vitro* drug release profile of Captopril from different ethosomal formulation

S.No	Time (h)	F_1	F_2	F_3	F_4	F_5
1	0	0	0	0	0	0
2	2	3.5±0.21	4.08±0.98	6.11±0.25	4±2.34	2.13±4.25
3	4	7.13±1.23	6.99±2.31	11.92±0.64	7.52±6.21	6.33±5.21
4	6	11.91±3.25	15.53±2.01	16.79±2.45	16.33±2.54	10.95±0.21
5	8	18.38±2.56	22.72±0.64	23.27±3.01	23.54±0.45	18.22±2.01
6	10	23.21±1.65	26.47±0.59	30.38±1.02	28.02±0.12	25.8±0.32
7	12	30.15±0.22	34.85±2.56	38.43±0.21	36.37±2.13	30.39±0.21
8	14	35.1±0.36	41.52±0.48	45.69±2.02	40.95±0.56	37.33±0.64
9	16	42.76±1.78	47.06±0.56	53.43±5.31	46.49±0.23	43.98±2.12
10	18	51.62±2.98	53.64±0.21	58.68±6.02	55.17±1.03	50.06±1.23
11	20	55.57±0.15	63.54±0.25	66.52±0.21	62.89±3.21	54.81±1.22
12	22	64.82±0.48	70.89±0.23	73.14±0.36	70.14±0.21	60.82±3.01
13	24	68.46±0.23	74.73±2.06	79.78±0.60	75.27±2.05	69.92±1.59

Table No.5: Total percentage release of captopril from different gel formulations

S.No	Gel formulation	Concentration of carbopol (%w/w)	Percentage cumulative release ± SD
1	F3-G1	1.0	72.54±1.45%
2	F3-G2	1.5	**85.21±1.23%**
3	F3-G3	2.0	64.45±0.21%

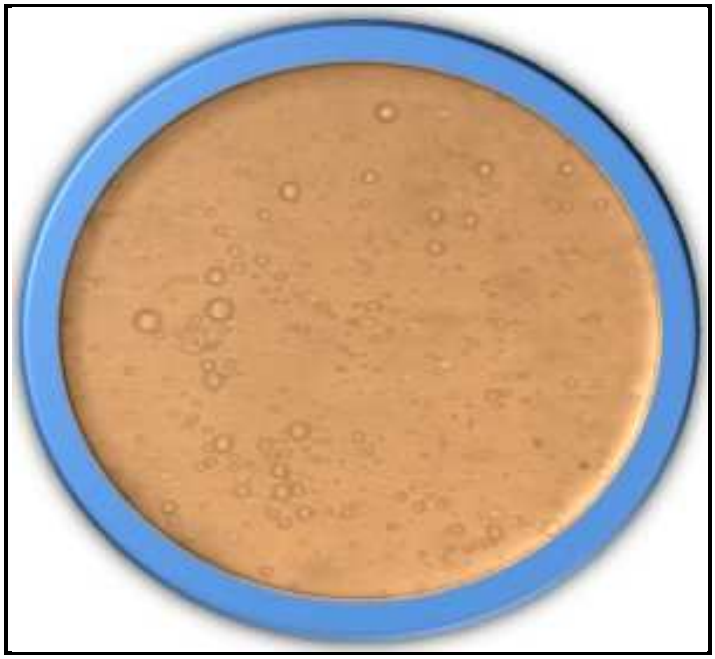

Figure No.1: A photograph of F3 formulation

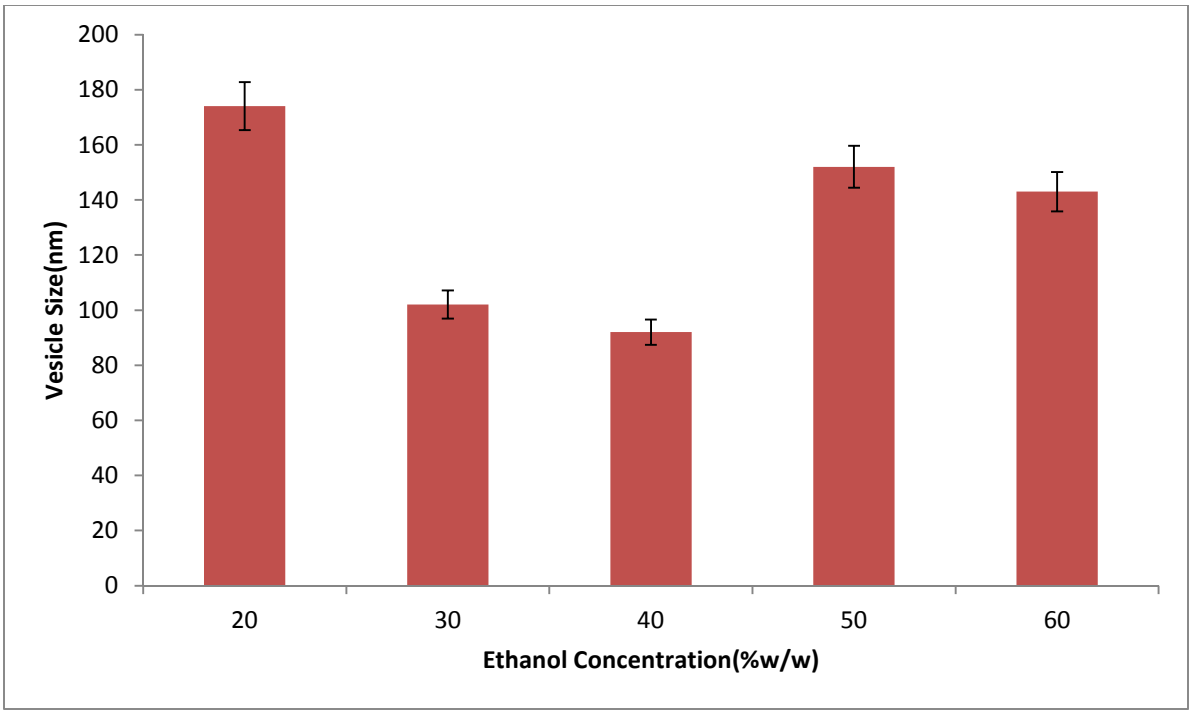

Figure No.2: Effect of ethanol concentration on size of ethosomal vesicles

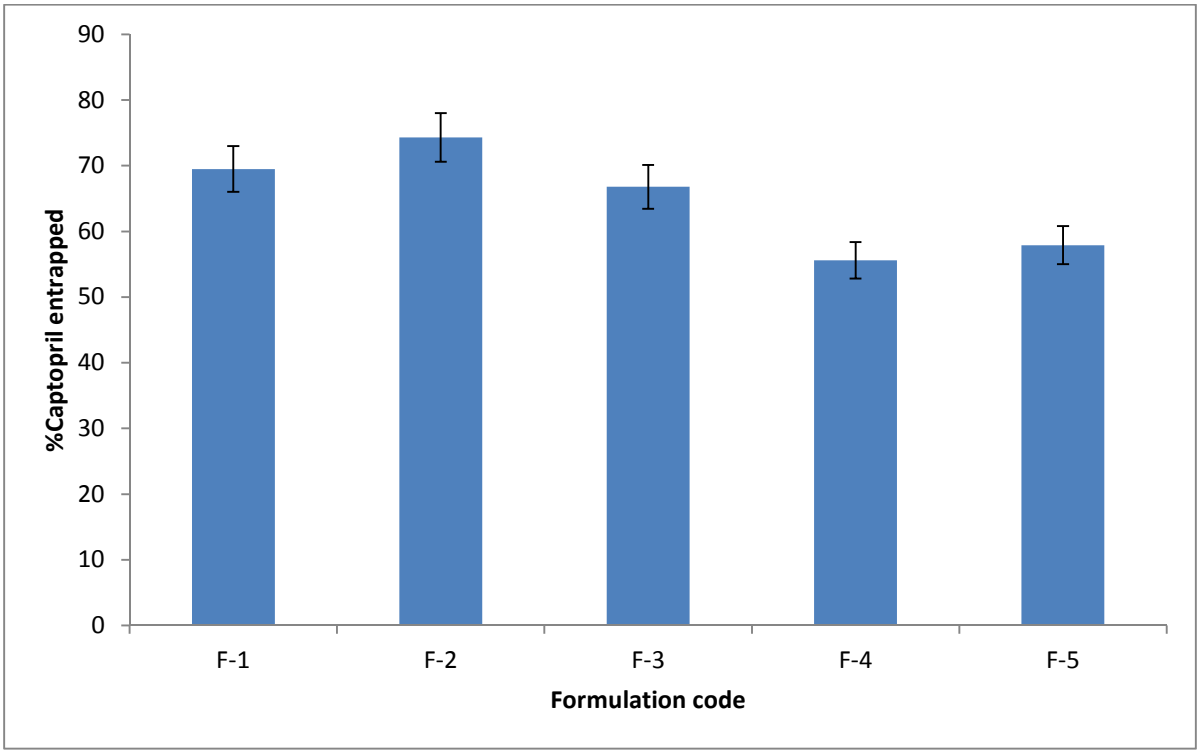

Figure No.3: Percentage entrapment efficiencies of different ethosomal vesicles

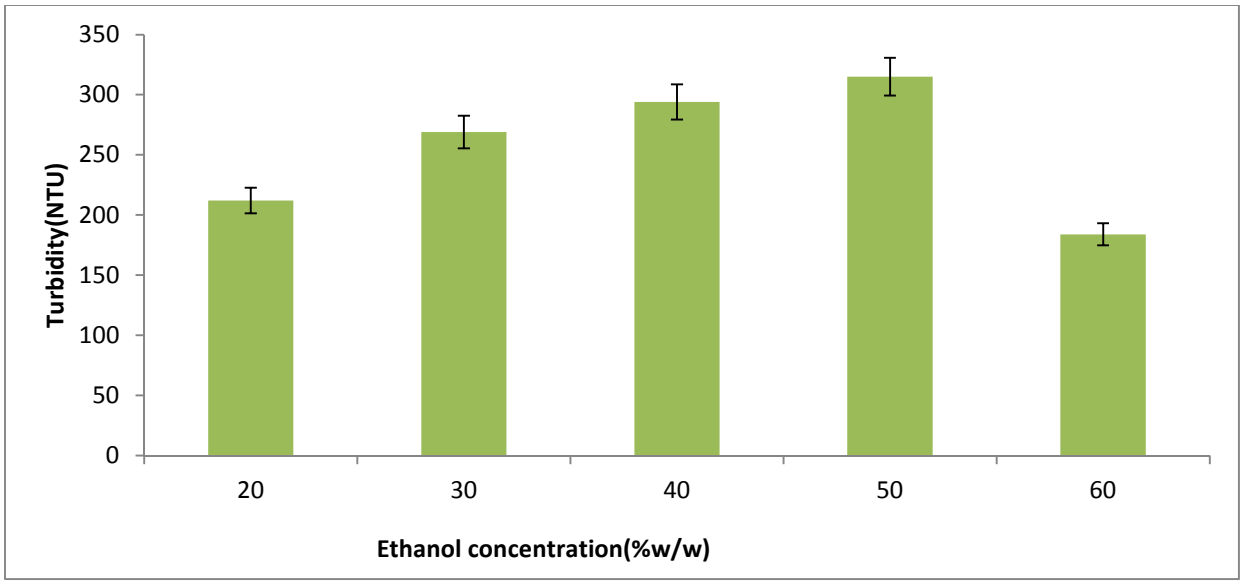

Figure No.4: Turbidity measurement of various ethosomal formulations by varying ethanol concentration

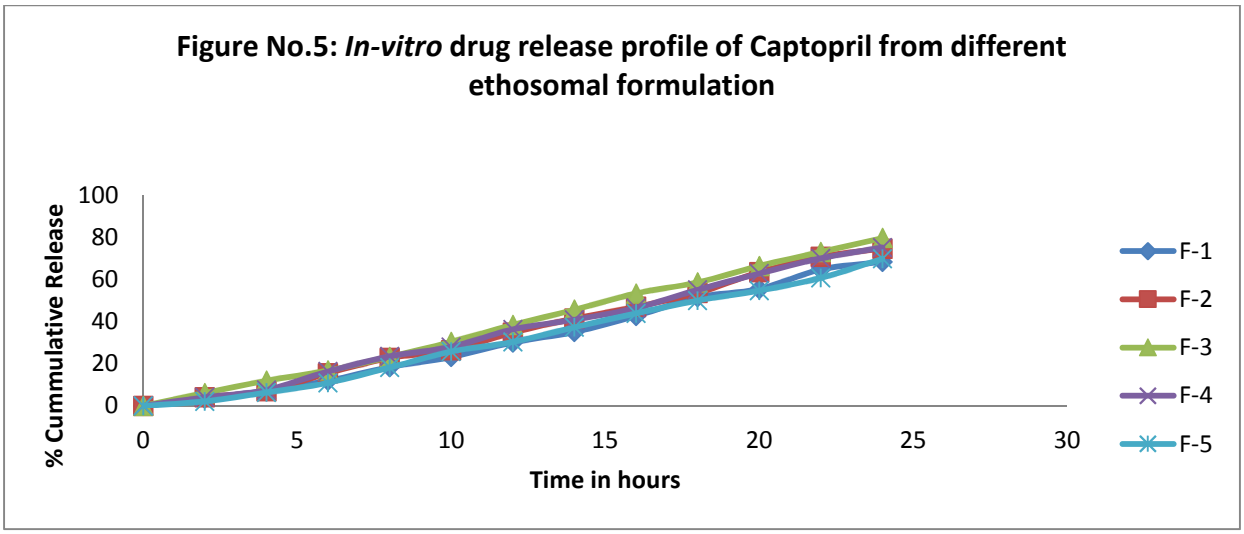

Figure No.5: *In-vitro* drug release profile of Captopril from different ethosomal formulation

CONCLUTION

Transdermal route is promising alternative to drug delivery for systemic effect. An attempt was made to formulate the highly efficient ethosomal drug delivery system and Captopril is used as model drug. The method described by Touitou *et al.*, v/v ethanol as the best formulation considering all other aspects. The highest value of transdermal flux for ethosomes containing 40% v/v ethanol is the (2000) was employed for the preparation of various ethosomal formulations containing different concentration of ethanol (20% to 60%). The sizes of vesicles were found in the formulation F3 to be in the range of 92±9.0nm. While comparing the entrapment efficiency, ethosomes containing 40% indication of complete and rapid penetration through the skin may be because of tiny vesicular size. This is an encouraging observation for drugs,

which are poorly absorbed from skin. All the formulation of ethosomes showed a zero order release for *in-vitro* release studies.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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