



**“Statistically optimized binary ethosomal gel of Carvedilol: allevialates hypertension in male Wistar albino rats”**

**Thota Sunil Kumar<sup>2</sup> and Ananda Kumar Chettupalli<sup>\*1</sup>**

2. Department of Pharmaceutics, University college of Pharmaceutical Sciences (UCPSc), Kakatiya university, Vidyananyapuri, Hanamakonda, Warangal, Telangana 506009, India.  
[sunithasunilthota@gmail.com](mailto:sunithasunilthota@gmail.com), +91- 9849237235, +91- 9502020950.

\* Department of Pharmaceutics, School of Pharmacy, Anurag Group of Institution, Venkatapur, Medchal, Hyderabad-500088, Telangana, India.- [anandpharmacy@cvsr.ac.in](mailto:anandpharmacy@cvsr.ac.in) +91- 9849922424, +91- 6303132567.

*Received: / Accepted: / Published:*

---

**Abstract:** The current study entails novel advanced formulation approach binary ethosomal gel containing Carvedilol and evaluating the optimized formulation for hypertension. Carvedilol is an antihypertensive agent, which undergoes hepatic metabolism and shows poor bioavailability of just 20 %. Carvedilol binary ethosomal suspension was optimized using central composite design (CCD). In which quantities of lipid (A), ethanol (b), and propylene glycol (C) were selected independent variables; vesicle size (Y1), entrapment efficiency (Y2) and cumulative %drug release (Y3) were selected as dependent variables. The composition of optimum formulation was found as 2 % of lipid (Soya lecithin), 20 % of ethanol, and 10 % of propylene glycol. The optimized binary ethosomal suspension (OBE) had shown a vesicle size, poly dispersable index, ztapotential, cumulative % drug release, and %entrapment efficiency of  $130\pm 1.72\text{nm}$ ,  $0.230\pm 0.03$ ,  $-31\pm 1.8\text{MV}$ ,  $97.89\pm 3.7\%$ , and  $99\pm 2.96\%$  respectively. This was further evaluated and compared with liposomes. Scanning electron microscopy studies revealed that OBE was in spherical shape. The OBE was converted to hydrogel and evaluated for rheological properties. The OBE- Gel (OBE-G) attained mean transdermal flux of  $0.0644\pm 0.002\text{ mg/cm}^2\cdot\text{h}$  through rat skin. This formulation showed a substantial and constant decrease

in blood pressure, for up to 24 h. The OBE-G was found to be effective, with reduction in blood pressure by virtue of better permeation through rat skin. In conclusion, OBE-G accentuate the transdermal flux and the results obtained encouraged its use for hypertension treatment.

**Keywords:** Carvedilol; Central composite design; hydrogel, hypertension.

## 1. Introduction

Transdermal drug delivery offers benefits over other routes of administration, which includes avoidance of pre-systemic metabolism and betterment of compliance. Unfortunately, the formidable barrier characteristics of stratum corneum (SC) present a significant obstacle for most drugs to be delivered through it. To surmount this barrier, new approaches of advanced vesicular carriers have been specially designed for the efficient delivery of active pharmaceutical ingredient (API) with different physicochemical properties across the skin. [1] [2].

Ethosomal systems are lipid vesicular carriers containing a high percentage of ethanol, probably this makes the vesicle membrane leaky, further leads to decrease in entrapment efficiency and stability [3] [4]. Therefore, to enhance stability, binary ethosomes have been developed. Which comprised of ethanol and propylene glycol (PG)? Binary ethosomes have an intact spherical shape

with a lipid bilayer. It can penetrate easily through SC and increase the amount of drug delivery effectively [5][6]. Further, for improving the consistency of the final dosage form, OBE was converted to hydro gel making it convenient for application on the skin surface of the patient.

Carvedilol is rapidly absorbed after oral administration, however, it possesses low oral bioavailability (about 20%) and high lipophilicity (Log P = 4.1) attributed to its poor dissolution (biopharmaceutical classification system (BCS class II) and extensive pre systemic metabolism [6][7]. The incorporation of the drug into lipid carriers leads to enhancement of bioavailability. Various formulation approaches like solid lipid nanoparticles (SLNs) and nasal microspheres have been employed to circumnavigate the hepatic route in order to increase carvedilol bioavailability [7].

Statistical designs help in optimization of formulation under a given set of conditions and

pre-determined goals. Among the various designs available, the central composite design (CCD) is a popular form of response surface methodology (RSM) which has been extensively employed in optimization and identifying the best formulation. This design is considered to be efficient in estimating the influence of individual variables (main effects) and their interaction

effects. Thus, CCD was employed for optimizing quality attributes of the prepared vesicles. [8] [9].

Thus, the scope of the present work was to evaluate the OBE-G of carvedilol developed using a statistical approach like Central composite design. Furthermore, the work also investigates the enhancement of therapeutic potential of OBE-G using *ex-vivo* permeation and pharmacodynamic studies. [10] [11].

## 2. Results and Discussion

### Experimental design optimization and response surface approach

#### Statistical analysis

The results implied that this system was colossally swayed by the amount of lipid, ethanol, and PG which resulted in small vesicle sizes, high % EE and high cumulative % drug release of the prepared ethosomes. The regression equations (4)–(6) were obtained using DoE.

$Y1 = +328.21 - 34.45 A + 302.50 B - 87.68 C - 143.50 AB - 80.50 AC + 16.00 BC + 127.57 A^2 + 136.05 B^2 + 5.24 C^2$

$Y2 = +95.43 + 7.59 A - 9.87 B + 2.15 C + 13.21 AB + 4.10 AC - 4.88 BC - 8.90 A^2 - 8.02 B^2 + 0.8494 C^2$

$Y3 = +94.98 + 4.71 A - 7.72 B + 2.63 C + 8.21 AB + 2.95 AC - 0.8025 BC - 1.23 A^2 - 4.21 B^2 - 0.7502 C^2$

In the regression equation the sign and value are the quantitative effect which represents the tendency and magnitude of the term's influence on the response. ANOVA for the responses indicated that the quadratic regression model was significant and valid for each of the responses Y1 ( $p < 0.0001$ ), Y2 ( $p < 0.0001$ ) and Y3 ( $p < 0.0001$ ) and hence was appropriate to represent the observed data, respectively which are shown in Table 3. The observed  $R^2$  values for the dependent responses are 0.9890, 0.9887 and 0.9662. When the observed value of  $R^2$  was at least 0.80, it implied a good correlation and was found in all cases, indicating a good fit by the model (41). The  $R^2$  values, for size (Y1) is 0.9790, % EE (Y2) is 0.9785 and Cumulative %

DR (Y3) is 0.9358 are high and advocated the significance of the model. The  $R^2$  Pre values, for size (Y1) are 0.9203, % EE (Y2) is 0.9318 and Cumulative % DR (Y3) is 0.9025, given by the model, which indicated a correlation between the predicted and observed values. Hence, the response model of this system is highly suitable for the selected responses. By using RSM the independent variables and their interactions on dependent responses were graphically represented by 3D surface plots. The effect of the amount of lipid, the amount of ethanol and the amount of PG on vesicle size, % EE and cumulative % DR are represented in Fig 1.

### Optimization of independent variable and validation

Further Optimization to probe the optimal formula of ethosomal suspension with desirable characteristics. This depended on the prescriptive criteria of minimum vesicle size, maximum % EE and maximum cumulative % DR. The composition of the optimum formulation was found as 2 % of lipid (Soya lecithin), 20 % of ethanol and 10 % of PG. At these levels, the predicted values of Y1 (vesicle size), Y2 (% EE) and Y3 (Cumulative % drug

release) were 130 nm, 99 %, and 99.98 % respectively.

A new batch of ethosomal suspension according to the optimal formulation was formulated in order to confirm the predicted model,. The observed OBE had shown a vesicle size, PDI, ZP, Cumulative % drug release and % EE of  $130\pm 9.72\text{nm}$ ,  $0.230\pm 0.03$ ,  $-31\pm 3.8\text{Mv}$ ,  $99.98\pm 3.7\%$  and  $99\pm 2.08\%$  respectively. A comparison between the results indicated the reliability of CCD in predicting a desirable ethosomal formulation.

### Solid-state characterization

#### Fourier transform infrared (FT-IR)

FTIR spectra of Carvedilol (Pure API), lipid, cholesterol, ethanol, PG, OBE, Carbopol, Triethanolamine, and OBE-G are shown in Fig 2. FTIR spectrum of Carvedilol showed peaks at  $3342.89\text{ cm}^{-1}$  which belongs to -N-H stretching,  $2922.72\text{ cm}^{-1}$  which belong to -C-H stretching,  $1443\text{ cm}^{-1}$  which belong to C-C stretching,  $1630\text{ cm}^{-1}$  and  $1607\text{ cm}^{-1}$  belong to C=C stretching and  $1347\text{ cm}^{-1}$  to  $1251\text{ cm}^{-1}$  belong to C-N stretching. All these characteristic peaks of Carvedilol were present in OBE and also it was found that there was no absence of any functional

peaks of Carvedilol in OBE and OBE-G spectrum. Hence the selected carriers were found to be compatible in entrapping the selected Carvedilol without any mutual interactions and remained stable during ethosomal formulation.

### Surface morphology using SEM

The OBE and liposomes were studied for surface morphology at 30.0 kV and 12.0 kV magnification using SEM. The morphology of nanoparticles was found to be nearly spherical in shape, and exhibited good PDI shown in Fig 3 A, B.

### Evaluation of topical gels

Control gel was prepared by adding Carbopol 934 (1% w/w) and drug to the water. The objective was to get an optimized gel formulation for topical application. The OBE-G possessed mean viscosity (cp), pH, Spreadability ( $\text{g.cm.sec}^{-1}$ ), and assay of  $2960 \pm 58$ cp,  $6.4 \pm 0.25$ ,  $5.61 \pm 0.08 \text{g.cm.sec}^{-1}$ , and  $99.88 \pm 2.58$  % respectively. The C-G possessed mean viscosity (cp), pH, Spreadability ( $\text{g.cm.sec}^{-1}$ ), and assay of  $2974 \pm 48$ cp,  $5.8 \pm 0.25$ ,  $5.38 \pm 0.08$ ,  $\text{g.cm.sec}^{-1}$ , and  $100 \pm 1.85$  % respectively. The results of the gels suggests that the pH of gel formulations were lies in normal pH range of skin (5.5-6.5)

and had not produced any skin irritation when applied to skin. The preparation was viscous and has low Spreadability. The assay of the gels was found to be more than 99%.

### *In-vitro* and *ex-vivo* Drug Permeation Studies

*In-vitro* drug permeation through dialysis membrane and *ex-vivo* drug permeation through rat skin were estimated and showed in Fig 4 A, B, C. About 50 % of the drug was permeated in 10h in case of OBE and 33 % in the case of the Liposomal formulation. This result indicated that the drug from OBE formulation permeated faster over the liposomal formulation, probably due to low vesicle size which increased permeability through the dialysis membrane.

The OBE-G showed more than 50% drug permeation in 16 h during *ex-vivo* permeation studies. Drug from OBE and OBE-G formulation was permeated relatively faster both *in-vitro* and *ex-vivo* studies when compared to liposomes and C-G. OBE and OBE-G showed the maximum flux than other formulations. From the physical characters, % EE, *in-vitro* drug permeation and *ex-vivo* permeation studies, formulation OBE-G was considered as better formulation.

### Pharmacodynamic study

The antihypertensive effect of OBE- G formulation was studied in comparison to OBE, liposomes and marketed formulation in the rat model. The hypertension was induced by 10 % oral MPA/Sodium solutions in rats. The systolic BP was measured and results were shown in Fig5 A, B. The transdermal administration of OBE-G controlled hypertension with the maximum effect up to 24 h, gradually until it was same as the initial value at 24 h. In the untreated group, there was no decrease in the systolic BP observed up to 24 h after the hypertension induction due to the effect of MPA/Sodium. In normal rat group, normal systolic BP was observed. From *ex-vivo*, the drug permeated from OBE-G was up to 28 h in comparison to C-G was observed and this clearly indicated that the OBE-G had permeated the drug gradually over a period of 24 h. Oral OBE suspension acted gradually (10 h in case of MPA induced and 6 h in case of sodium-

induced) but, then its effect dropped off, whereas the OBE-G could not decrease the BP greatly in the initial phase when compared with the other formulations. The marketed formulation reduced the BP greatly in the initial phase when compared to other formulations but, then its effect dropped off after 2 h both in MPA and sodium-induced hypertension. Since the administration of designed ethosomal gel (OBE-G) resulted in sustained and continued drug permeation for 24 h and beyond (from *ex-vivo* studies), to control hypertension throughout the 24 h period. Obviously, the obtained optimized formulation was capable of surmounting the shortcomings of oral administration of Carvedilol, such as low bioavailability and high first-pass metabolism. Further, it becomes a clinical advantage in controlling hypertension slowly, steadily and for an extended period by designing the drugs in ethosomal gel formulation.

**Table 1.** Experimental strategy of CCD with coded composition values of process variables along with the observed values.

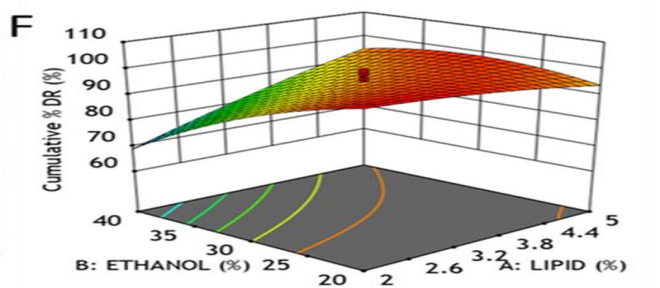
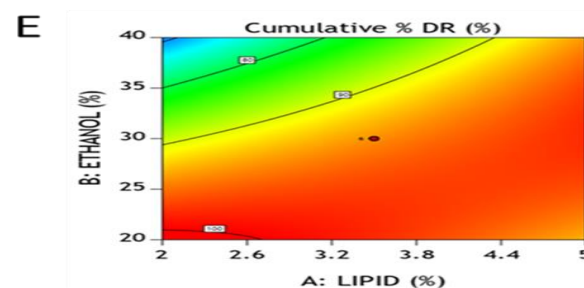
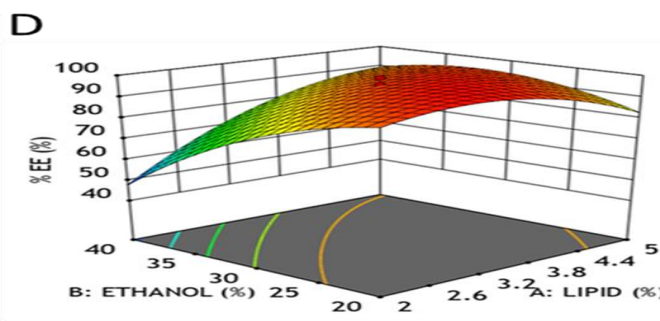
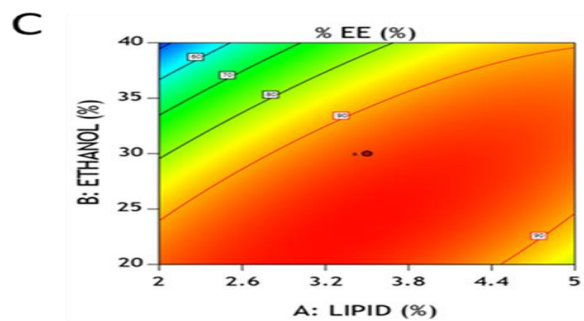
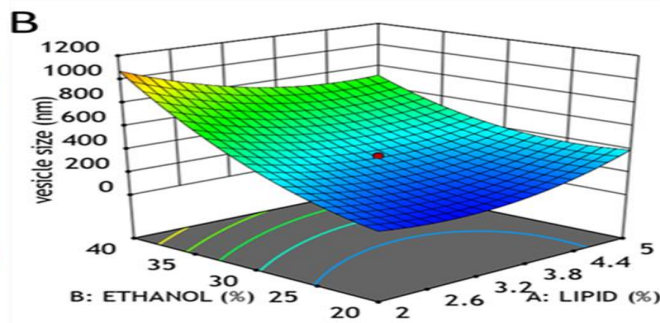
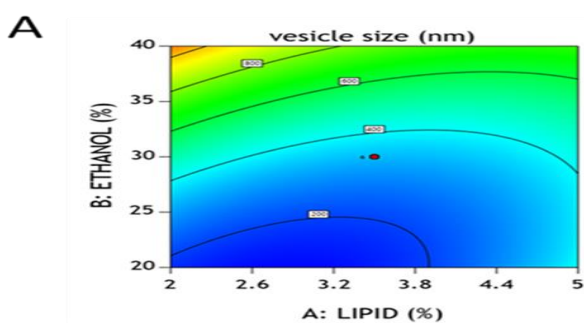
Formulation code	Independent variables			Dependent variables		
	A:LIPID %	B:ETHANOL %	C:PROPYLENE GLYCOL %	Y1: Vesicle Size (nm)	Y2: % EE (%)	Y3: Cumulative % DR (%)
1	0	0	0	140	91.8	99
2	-1	-1	-1	280	89.56	95.87
3	1	-1	1	600	94.09	98.74
4	0	0	0	200	95.82	98.82
5	0	0	0	550	94.41	87.5
6	-1.68	0	0	321	95.24	93.35
7	0	-1.68	0	1200	52.67	71.08
8	1	1	-1	550	71.82	88.23
9	0	0	0	345	98.87	92.52
10	0	0	0	315	94.25	90.58
11	0	0	1.68	318	95.46	98
12	0	1.68	0	1050	56.74	69.29
13	1.68	0	0	800	55.45	85.26
14	1	1	1	311	95.78	99.24
15	-1	1	-1	632	81.79	98.51
16	-1	1	1	130	99	99.89
17	0	0	-1.68	350	93.56	96.07
18	0	0	0	868	90	89.28
19	1	-1	-1	1086	44.82	64.89
20	-1	-1	1	190	98	99

Table 2-Regression values of the selected responses during optimization.

Model	Y1 (Vesicle size)			Y2 (% EE)			Y3 (Cumulative % DR)		
	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>
Linear	0.6611	0.5976	0.4484	0.3675	0.2489	0.0766	0.5597	0.4771	0.2359
2FI	0.7666	0.6588	0.5264	0.6574	0.4993	0.3302	0.8434	0.7711	0.6347
Quadratic	0.9890	0.9790	0.9203	0.9887	0.9785	0.9318	0.9662	0.9358	0.9025
<i>p</i> value		0.0001			0.0001			0.0001	

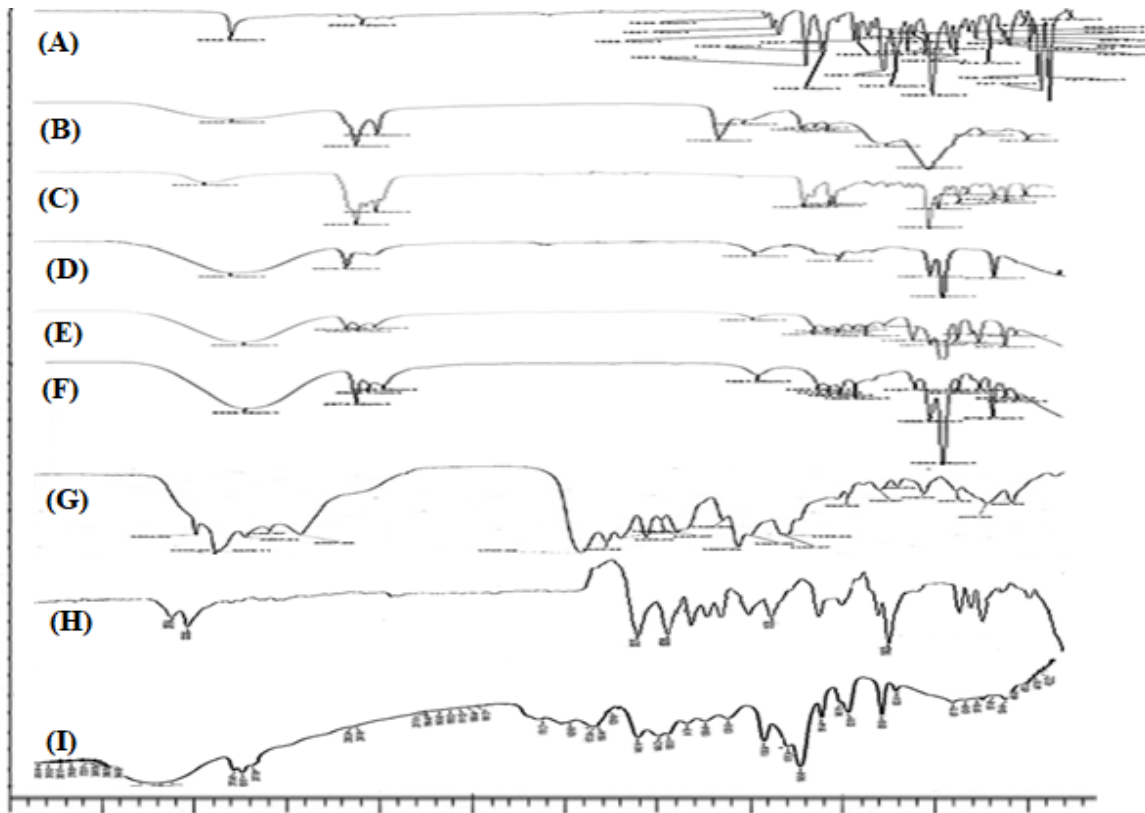
Table 3-ANOVA of optimized quadratic model.

Parameter	Source	DF	Sum of squares	Mean of squares	F Value	p Value
Vesicle size	Model	9	2.051	2.279	99.65	<0.0001
	Residual	10	22866.43	2286.64		
	Lack of fit	5	21497.1	4299.42		0.0045
	Pure error	5	1369.33	273.87		
% EE	Model	9	5869.50	652.17	97.24	<0.0001
	Residual	10	67.07	6.71		
	Lack of fit	5	50.24	10.05		0.0029
	Pure error	5	16.83	3.37		
Cumulative % DR	Model	9	2090.19	232.24	31.76	<0.0001
	Residual	10	73.13	7.31		
	Lack of fit	5	16.61	3.32	16.85	0.0105
	Pure error	5	56.52	11.30		





**Figure 1.**Contour plots and response plots showing the interactive effects (i) amount of Lipid (A) and amount of ethanol (B) on vesicle size (Y1) (A & B) (ii) amount of Lipid (A) and amount of ethanol (B) on %EE (Y2) (C & D) (iii) amount of Lipid (A) and amount of ethanol (B) on cumulative % drug release (Y3) (E & F).



**Figure 2.**FTIR spectrums of (A) Carvedilol pure drug, (B) Lipid ( Soya Lecithin), (C) Cholesterol, (D) Ethanol, (E) PG, (F) OBE, (G) Carbopol, (H) Triethanolamine, and (I) OBE- G.

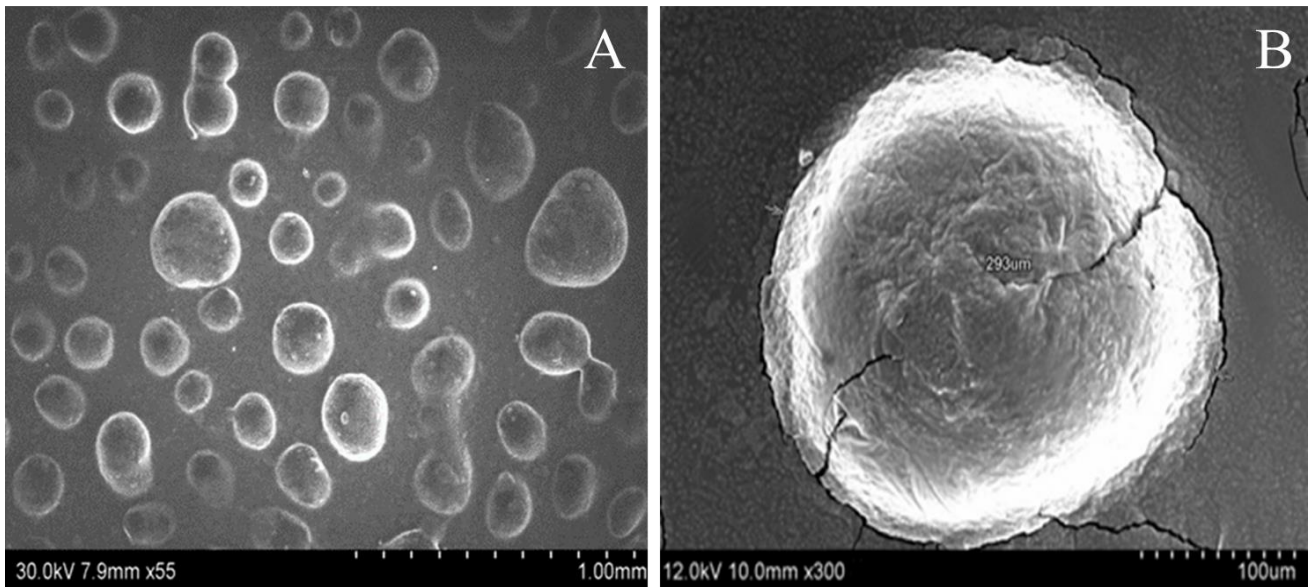


Figure 3. SEM images of A. OBE-G B. Liposome

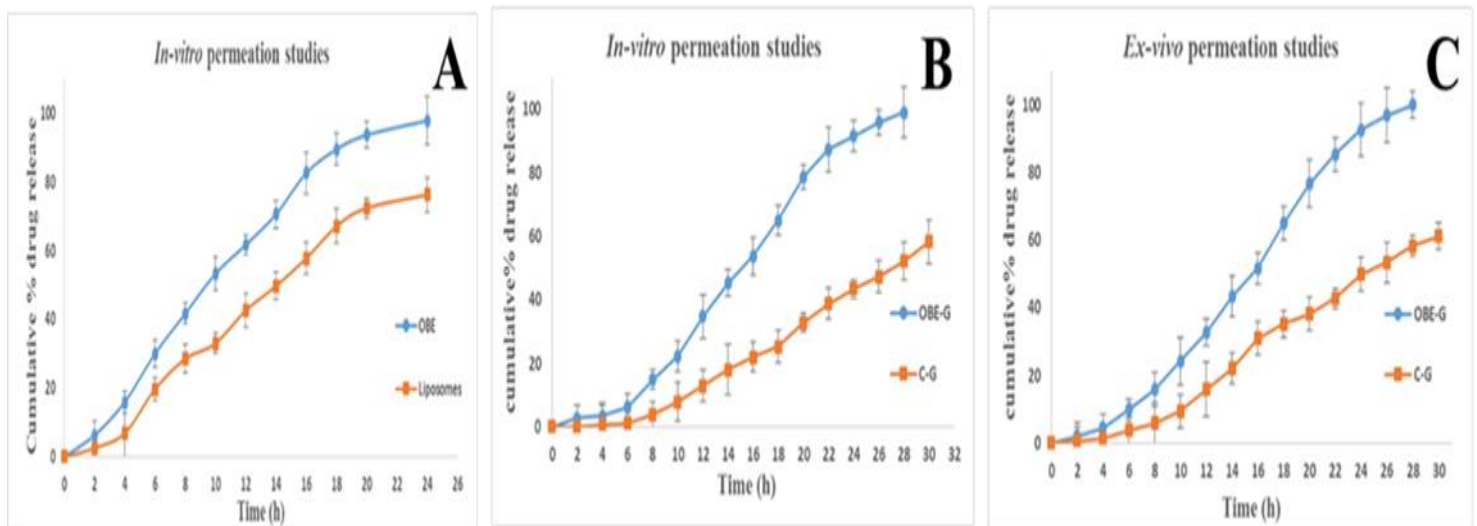
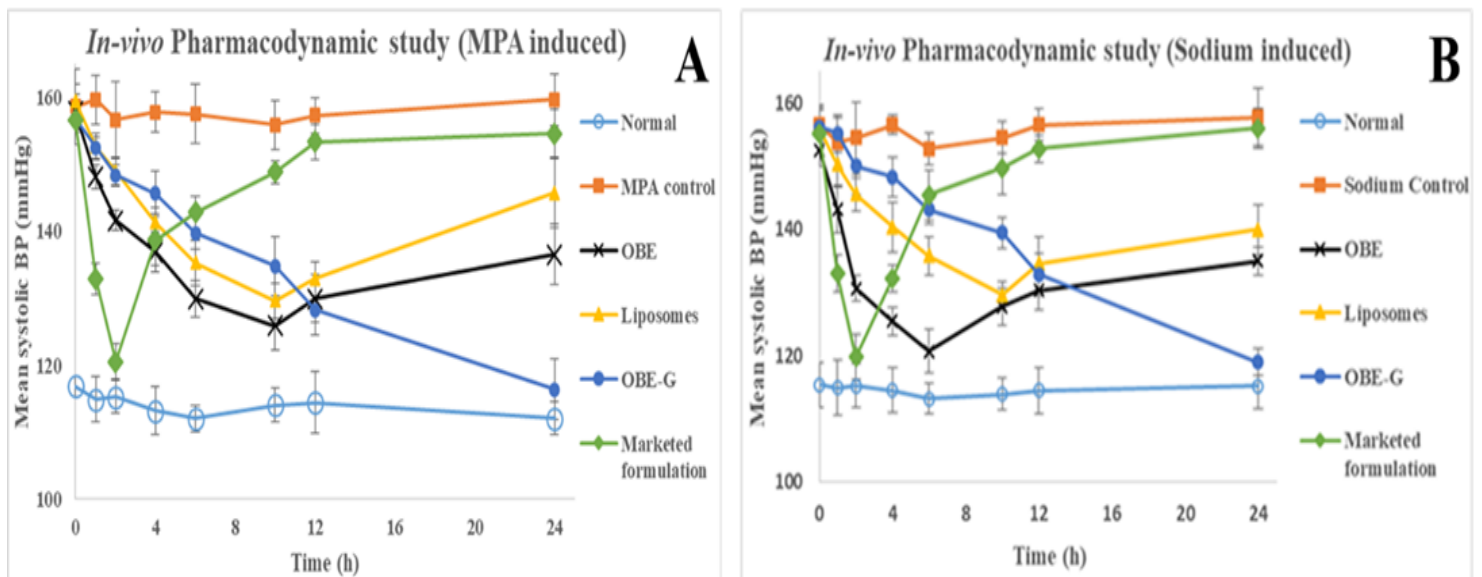


Figure 4. In-vitro and Ex-vivo permeation studies



**Figure 5.**Pharmaco-dynamic profiles of different formulation types of Carvedilol in rats.

### 3. Materials and Methods

Carvedilol gift was purchased from Chandra Laboratories, Hyderabad. Soya lecithin, Propylene glycol (PG), Triethanolamine and Carbopol-934 has purchased from Research lab Fine Chem. Industries, Mumbai. Alcohol has purchased from Jiangsu Huaxi International Trade Co.Ltd, China. Cholesterol was purchased from Merck Ltd, Mumbai. Ultrapure water has purchased from Cortex laboratories, Hyderabad. Centrisart filters (molecular weight cut off 20 000) were purchased from Sartorius, Goettingen, Germany. All other chemicals used were of analytical grade and solvents were of HPLC grade.

#### **High performance liquid chromatography (HPLC) analysis**

The high performance liquid chromatography (HPLC) system (Shimadzu, Japan) consisted of an LC-10AT solvent module, and a model SPD-10A, UV-Visible Spectrophotometric detector with LC 10

software. The chromatographic separation was performed by using Kromosil reversed-phase C18, 150 mm× 6.0 mm, 5 µm column. The mobile phase consisted of acetonitrile, 15 mM ortho-phosphoric acid (37:63), and 0.25 v/v% triethylamine mixture, and was adjusted to pH 2.5 with ortho-phosphoric acid volume of injection was 20 µl. The elute was monitored at wavelength 238 nm with a flow rate of 1 mL/min and the sample run duration was 12 min. [12].

#### **Experimental design for the formulation of binary ethosomes**

In this study, a CCD was used to optimize the formulation variables of Carvedilol binary ethosomal suspension containing three factors and was evaluated at three levels. The amount of drug (25 mg) and cholesterol (5 mg) was kept constant and the experimental trials were performed. Vesicle size (Y1), % entrapment efficiency (% EE) (Y2) and Cumulative % drug release (%) (Y3) were included as dependable responses. The

experiments were designed by using DoE software (Version 11, Stat-Ease Inc., and Minneapolis, MN) and the layout of the design is shown in Table 1. The DoE software was used to give information on the critical values required to achieve the desired response and also all the possible interactions of the selected independent variables on the dependent variables. The response surface method normally approximates the correlation function as a full quadratic equation (Equation (1)) and is based on the experimental design [13]:

$$Y = B_0 + \sum_{i=1}^2 B_i X_i + \sum_{i < j}^2 B_{ij} X_i X_j + \sum_{i=1}^2 B_{ij} X_i^2 + \epsilon$$

Where Y is a response equation applicable for the vesicle size, % EE and cumulative % drug release; X<sub>i</sub> terms include independent variables (X = A (amount of lipid), B (amount of ethanol), and C (amount of PG) ranging from (-1 ≤ X ≤ 1)). B<sub>i</sub> terms are the equation coefficients related to the main factor. ε is the experimental error. The analysis of variance (ANOVA) and statistical

data analysis were used to know the significant effect of the factors and their interactions.

### Preparation of binary ethosomal suspension

The carvedilol binary ethosomal suspension was formulated by adding 25mg of carvedilol, soya lecithin, and 5 mg Cholesterol to binary alcoholic phase (comprised of ethanol and PG) under continuous stirring. Then it was heated up to 30°C and was added drop wise to an aqueous part by stirring it at 700rpm for 5min followed by sonication. At last the binary ethosomal suspension was stored under refrigeration until further characterization [14] [15] [16].

### Assay (%)

To determine assay (%), the formulation was dissolved in chloroform/methanol (1:1) mixture and the final dilution was made with the mobile phase for determining Carvedilol content by HPLC [17].

### % Percentage entrapment efficiency

To determine Percentage entrapment efficiency (% EE), centrifugation was performed using Centrisart tubes at 8000 rpm for 30 min. The % EE of the system was determined by measuring the concentration of free drug in the

dispersion medium using HPLC, along with the following formula [18] [19].

% Entrapment Efficiency (% EE)

$$= \left[ \frac{A_{total} - A_{unentrapped}}{A_{total}} \right] \times 100$$

$A_{total}$  =total amount of Carvedilol in ethosomal suspension;

$A_{unentrapped}$  =unentrapped Carvedilol in ethosomal suspension.

### Stability studies

OBE was stored at refrigerated temperature (4°C) and room temperatures (30°C) for three months. The mean average vesicle size, PDI, zeta potential, % EE and % Assay were determined periodically after zero-day (Initial hour), 2W, 1M, 2M, and 3M. The number of samples estimated was in triplicate [20] [21] [22].

### Solid-state characterization

#### Fourier transform infrared (FT-IR) spectroscopy

To investigate any physical interactions between the drug and the excipients used in the formulation, compatibility studies were carried out at room temperature by Fourier transform infrared spectroscopy (FTIR) (Model- 200,

Thermo Electron, Shimadzu, Japan). The pure drug (Carvedilol), OBE and OBE-G and all excipients used were subjected to FTIR studies, which were analyzed by the KBr pellet technique. The scanning range was 400-4000  $\text{cm}^{-1}$  and the resolution was 4  $\text{cm}^{-1}$  [23].

### Surface morphology using SEM

OBE surface morphology was studied by using scanning electron microscopy. The sample of formulations has first adhered to the carbon-coated metallic stub and was sputter coated with Platinum coating machine (JFC-1600 Auto fine coater, JEOL, Tokyo, Japan) and mounted in SEM (JSM-6510LA, JEOL, Tokyo, Japan) for imaging at high vacuum [24] [25] [26].

### Preparation of carvedilol classical liposomes

Liposomes were prepared by dissolving phospholipids 2% w/w, drug 25mg and cholesterol 5mg with a small quantity of chloroform in a round-bottomed flask. Chloroform was removed by rota evaporator to form a thin film inside the flask and hydrated with diluted with hydrochloric acid which was made up to 20gm w/w with water [17].

### Preparation of hydro gels

The accurately weighed quantity of Carbopol 934 (1% w/w) was added to a beaker containing OBE and was kept aside for complete hydration of the polymer. Further, the gel was prepared by adding 0.5 mL of Triethanolamine as a neutralizer and was characterized [27] [28] [29].

### Evaluation of Topical Gels

#### pH Measurements of prepared Gels

The pH was determined using a digital pH meter (Remi, Hyderabad, India). The pH of the gel was recorded by completely dipping the glass electrode into the gel system [30].

**Viscosity Measurement of Gel** The viscosity of formulated gel was determined using a High torque and low-temperature Brookfield (CAP 2000+L, frequency: 50/60Hz, USA (cone & plate)). The cone (No. 01) was used to measure the viscosity of preparations. About 500 mg sample was placed on the plate and allowed to settle for 5 minutes prior to taking the readings to achieve room temperature and the results were noted [31].

#### Skin irritation test

The study was carried out on nine Male Wister rats to evaluate and compare skin

irritation of OBE-G and blank ethosomal gel (without drug). The hairs of the dorsal portion were removed with the help of shaver. The rats were divided into three groups of 3 rats each as follows: Group 1: No application (Normal). Group 2: OBE-G. Group 3: blank ethosomal gel (without drug). OBE-G (500 mg) and blank ethosomal gel (500 mg) was applied on the hairless skin of the rats by uniform spreading within the area of 4cm<sup>2</sup>, respectively. The skin was observed for any indication of erythema or redness after a time interval of 24, 48 and 72 hrs [32] [33] [34].

#### Spreadability:

The determination of Spreadability was done by glass slide apparatus and modified wooden block. A weighed quantity of the gel was put over the movable pan with a slide of glass attached to it and was then placed on the fixed glass slide so that the gel was sandwiched in between the two glass slides for a duration of 5min. The continuous removal of weight was also done. The determination of Spreadability was done by using the formula [35]:

$$S=M/T$$

where, S= Spreadability in g/s, M= Mass in gms,  
T=Time in secs.

### ***In-vitro* permeation Studies**

The *in-vitro* permeation of OBE, liposomes, and hydrogels was determined by using vertical Franz-diffusion cell mounted with Dialysis membrane, which was soaked overnight in PBS (Phosphate Buffer Saline)(pH 7.4) prior to the release studies. During the experiment receptor compartment was maintained at a constant temperature of  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ , while the receptor medium was stirred constantly at 100 rpm. At predetermined time intervals, about 1 mL of aliquot samples were collected and replenished with the same volume of fresh medium. The cumulative % drug release through the membrane were estimated using HPLC and the obtained results were plotted as a function of time [36] [37].

### ***Ex-vivo* skin permeation studies**

The rats were sacrificed and to their abdomen depilatory paste were applied. A patch of hairless skin was excised from the back region of each sacrificed rat and the subcutaneous fat was removed and then washed with physiological saline. The excised skin was

carefully mounted between donor and receptor compartments of Franz- diffusion cells.

The *Ex-vivo* skin permeation capability of Carvedilol OBE-G and Control gel (C-G) (normal gel with the drug) was evaluated using Franz- diffusion cells. At predetermined time intervals, about 1 mL of aliquot samples were collected and replenished with same. Samples were analysed by HPLC as described above [38].

### **Pharmacodynamic study**

All these animal studies were conducted in animal house within the guidelines of Institutional Animal Ethical Committee and permission No.51/01/C/CPCSEA/2013/13.

Two different methods were performed in evaluating the pharmacodynamics activity of OBE and OBE-G in comparison with liposomes and marketed formulation. Each method has two groups they are untreated group i.e., normal and either Methyl Prednisolone Acetate (MPA) control or Sodium Control (hypertensive) and treated group i.e., OBE, OBE- G, liposomes, and marketed formulation (each of six), weighing 210–250 g were used in the study and allowed free access to standard laboratory diet and

drinking fluid (tap water or 10% Sodium solutions). The rats were trained to stay calm and non-aggressive state in the rat holder during BP measurement. Two weeks later, rats with a minimum mean systolic BP of 150–160mmHg were selected and given 10 mg/kg orally except OBE- G and C-G (10 mg/kg transdermal route) before initiating the study. Using the tail-cuff method (Bio-pack system inc. santabarbara, USA), systolic blood pressure (BP) was measured at different time intervals (0, 1, 2, 4, 6, 10, 12, and 24 h) for all groups [39] [40].

#### 4. Conclusions:

Carvedilol binary ethosomal suspension was prepared and optimized by using CCD. The

SEM images revealed that the OBE was nearly spherical showing good PDI and possessed smooth surface. From the FTIR studies, it was found that, the Carvedilol was compatible with selected carriers and excipients and it was stable during ethosomal formulation. The conducted skin irritation test and stability studies revealed that the OBE was safe and stable for three months. The pharmacodynamic efficacy carried out in rats showed a reduction in mean systolic BP upto 24 h from OBE- G. In nutshell, the results obtained depicts the increment in the therapeutic potential of OBE-G was suitable for transdermal drug delivery.

#### Acknowledgments

The authors report no conflicts of interest. The authors are alone responsible for the content and writing of this paper.

#### References and Notes

- [1] Jian-Ping Zhang, Yu-Hui Wei, Yan Zhou, Yu-Qing Li, and Xin-An Wu, Ethosomes, Binary Ethosomes and Transfersomes of Terbinafine Hydrochloride: A Comparative Study. Arch Pharm Res. 2012; 35: 109-17.
- [2] Ibrahim M Abdulbaqi, Yusrida Darwis, Nurzalina Abdul Karim Khan, Reem Abou Assi, and Arshad A Khan Ethosomal nanocarriers: the impact of constituents and Formulation techniques on ethosomal properties, in vivo studies, and clinical trials. Int J Nanomedicine. 2016; 11: 2279-304.
- [3] E. Touitou, N. Dayan, L. Bergelson, B. Godin, M. Eliaz, Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of Controlled Release. 2000; 65: 403– 18.



- [4] E. Touitou, B. Godin Ethosomes for skin delivery, *J. Drug Del. Sci. TECH.* 2007; 17: 303-8.
- [5] Xiaoliang Zhu, Ph.D., Fuli Li, MM, Xuebiao Peng, MD, and Kang Zeng, MM, Formulation and Evaluation of Lidocaine Base Ethosomes for Transdermal Delivery. [www.anesthesia-analgesia.org](http://www.anesthesia-analgesia.org). 2013; 117: 352-7.
- [6] Ines Castangia, Maria Letizia Manca, Pietro Matricardi Ana Catalan-Latorre, Amparo Nacher, Octavio Diez-Sales, Xavier Fernandez-Busquets, Anna Maria Fadda, Maria Manconi, Effects of ethanol and diclofenac on the organization of hydrogenated phosphatidylcholine bilayer vesicles and their ability as skin carriers, *J Mater Sci: Mater Med.* 2015; 26: 137.
- [7] Vinay Kumar Venishetty, Raghavendra Chede, Rojarani Komuravelli, Laxminarayana Adepu, Ramakrishna Sistla, Prakash V. Diwan, Design and evaluation of polymer coated carvedilol loaded solid lipid nanoparticles to improve the oral bioavailability: A novel strategy to avoid intraduodenal administration, *Colloids and Surfaces B: Biointerfaces.* 2012; 95: 1– 9.
- [8] Bhupinder Singh, Ramandeep Singh, Shantanu Bandyopadhyay, Rishi Kapil, Babita Garg, Optimized nanoemulsifying systems with enhanced bioavailability of carvedilol, *Colloids and Surfaces B: Biointerfaces.* 2013; 101: 465– 74.
- [9] Hitesh Dalvadi, Nikita Patel & Komal Parmar, Systematic development of DoE optimized Self-Microemulsifying Drug Delivery System of Zotepine, *J of Microencapsul.* 2017; 34: 308-18.
- [10] Abdul Ahad, Mohammad Raish, Abdullah M Al-Mohizea, Fahad I. Al-Jenoobi, Mohd Aftab Alam, Enhanced anti-inflammatory activity of carbopol loaded meloxicam nanoethosomes gel, *International Journal of Biological Macromolecules.* 2014; 67: 99–104.
- [11] Deepak Bisht, Devina Verma, Mohd. Aamir Mirza, Md. Khalid Anwer, Zeenat Iqbal, Development of ethosomal gel of ranolazine for improved topical delivery: In vitro and ex vivo evaluation, *Journal of Molecular Liquids.* 2017; 225: 475-81.
- [12] Gannu R, Vishnu YV, Kishan V, Rao YM, Development of carvedilol transdermal patches: evaluation of physicochemical, ex vivo and mechanical properties, *PDA J Pharm Sci Technol.* 2008; 62: 391-401.
- [13] Abdul Ahad, Mohd. Aqil, Kanchan Kohli, Yasmin Sultana, Mohd. Mujeeb, Enhanced transdermal delivery of an anti-hypertensive agent via nanoethosomes: Statistical optimization, characterization and pharmacokinetic assessment, *International Journal of Pharmaceutics.* 2013; 443: 26– 38.
- [14] Sagar S. Bhosale and Amelia M. Avachat, Design and development of ethosomal transdermal drug delivery system of valsartan with preclinical assessment in Wistar albino rats, *J Liposome Res.* 2013; 23(2): 119–25.
- [15] Tarek M. Ibrahim, Marwa H. Abdallah, Nagia A. El-Megrab & Hanan M. El-

- Nahas, Upgrading of dissolution and anti-hypertensive effect of Carvedilol via two combined approaches; self-emulsification and liquisolid techniques, *Drug Dev Ind Pharm.* 2018; 44: 873-85.
- [16] Zhang Y, Ng W, Hu J, Mussa SS, Ge Y, Xu H, Formulation and in vitro stability evaluation of ethosomal carbomer hydrogel for transdermal vaccine delivery, *Colloids surf B Biointerface.* 2018; 163: 184-91.
- [17] Mustafa M.A. Elsayed, Ossama Y. Abdallah, Viviane F. Naggar, Nawal M. Khalafallah, Deformable liposomes and ethosomes: Mechanism of enhanced skin delivery, *International Journal of Pharmaceutics.* 2006; 322: 60–6.
- [18] Yong-Tai Zhang<sup>Q1</sup>, Li-Na Shen, Zhong-Hua Wu, Ji-Hui Zhao, Nian-Ping Feng, Comparison of ethosomes and liposomes for skin delivery of psoralen 2 for psoriasis therapy, *Int J Pharm.* 2014; 471: 449-52.
- [19] Mahmood S, Mandal UK, Chatterjee B, Transdermal delivery of raloxifene HCl via ethosomal system: Formulation, advanced characterizations and pharmacokinetic evaluation. *Int J Pharm.* 2018; 542: 36-46.
- [20] Ahmed Khalid M. El-Say Bader M. Aljaeid Usama A. Fahmy Fathy I. Abd-Allah, Transdermal glimepiride delivery system based on optimized ethosomal nano-vesicles: Preparation, characterization, in vitro, ex vivo and clinical evaluation, *Int J Pharm.* 2016; 500: 245-54.
- [21] Gashin Zandi, Farzaneh Lotfipour, Saeed Ghanbarzadeh, Mahsa Medghalchi, Hamed Hamishehkar, A comparative study on the potentials of nanoliposomes and nanoethosomes for Fluconazole delivery, *Journal of Drug Delivery Science and Technology.* 2018; 44: 264-9.
- [22] Santosh Shelke, Sadhana Shahi, Kiran Jadhav, Dinesh Dhamecha, Roshan Tiwari, Hemlata Patil, Thermoreversible nanoethosomal gel for the intranasal delivery of Eletriptan hydrobromide, *J Mater Sci: Mater Med.* 2016; 27: 103-10
- [23] Moayad Hossaini Sadr and Hafezeh Nabipour Synthesis and identification of carvedilol nanoparticles by ultrasound method, *Journal of Nanostructure in Chemistry.* 2013; 3:26.
- [24] Yan Yan, Hefeng Zhang, Jinyao Sun, Pengchong Wang, Kai Dong, Yalin Dong, Jianfeng Xing, Enhanced transdermal delivery of sinomenine hydrochloride by ethosomes for anti-inflammatory treatment, *Journal of Drug Delivery Science and Technology.* 2016; 36: 201-7.
- [25] Yan Wo, Zheng Zhang, Yixin Zhang, Zhen Zhang, Kan Wang, Xiaohui Mao, Weijie Su, Ke Li, Daxiang Cui and Jun Chen, Enhanced in Vivo Delivery of 5-Fluorouracil by Ethosomal Gels in Rabbit Ear Hypertrophic Scar Model, *Int. J. Mol. Sci.* 2014; 15: 22786-800.

- [26] Udhumasha Ubaidulla, Molugu V.S. Reddy, Kumaresan Ruckmani, Farhan J. Ahmad, and Roop K. Khar, Transdermal Therapeutic System of Carvedilol: Effect of Hydrophilic and Hydrophobic Matrix on In Vitro and In Vivo Characteristics, *AAPS PharmSciTech*. 2007; 8: 1-8.
- [27] Syed Ahmed Iizhar, Ismail Ahmed Syed, Rukhsana Satar, Shakeel Ahmed Ansari, In Vitro assessment of pharmaceutical potential of ethosomes entrapped with Terbinafine Hydrochloride, *J Adv Res*. 2016; 7: 453-61.
- [28] Garg BJ, Garg NK, Beg S, Singh B, Katare OP, Nanosized ethosomes-based hydrogel formulations of methoxsalen for enhanced topical delivery against vitiligo: formulation optimization, in vitro evaluation and preclinical assessment, *J Drug Target*. 2016; 24: 233-46.
- [29] Sanjay J. Kshirsagar, Mangesh R, Bhalekar, and Santosh K, Mohapatra, Development and evaluation of carvedilol-loaded transdermal drug delivery system: In-vitro and in-vivo characterization study, *Drug Development and Industrial Pharmacy*. 2012; 38: 1530–7.
- [30] Bharadwaj. Snigdha, Garg Vipin Kumar, Sharma PK, Bansal Mayank and Kumar Nitin: “Recent advancement in transdermal drug delivery system”. *International Journal of Pharma Professionals*, 2011; 2(1): 247-54.
- [31] Rizwan. M, Aqil. M, Talegoankar. S, Azeem. A Sultana. Y, Ali. A. “Enhanced transdermal drug delivery techniques: an extensive review on patents”. *Recent Pat Drug Deliv&formul*. 2009, 3(2): 105-24.
- [32] Marto J, Vitor C, Guerreiro A, Severino C, Eleuterio C, Ascenso A, Simoes S, Ethosomes for enhanced skin delivery of griseofulvin, *Colloidal Surf B Biointerface*. 2016; 146: 16-23.
- [33] Behtash Oskuie S, Nasrollahi S, Afisi, Design, synthesis of novel vesicular systems using turpentine as a skin permeation enhancer, *Journal of Drug Delivery Science and Technology*. 2018; 43: 327-32.
- [34] GM El Maghraby, AC Williams, Vesicular systems for delivering conventional small organic molecules and larger macromolecules to and through human skin, *Expert Opin. Drug Deliv*. 2009; 6: 149-63.
- [35] Vidya Sabale, Harish Kunjwani, Prafulla Sabale, Formulation and in vitro evaluation of the topical antiageing preparation of the fruit of *Benincasa hispida*, *J Ayurveda Integr Med*. 2011; 2: 124-8.
- [36] Harneet Marwah, Tarun Garg, Amit K. Goyal, and Goutam Rath, Permeation enhancer strategies in transdermal drug delivery, *Drug Deliv*. 2016; 23: 564-78.

- [37] Guiling Li, Yating Fan, Chao Fan, Xinru Li, Xiaoning Wang, Mei Li, Yan Liu, Tacrolimus-loaded ethosomes: Physicochemical characterization and in vivo evaluation, *European Journal of Pharmaceutics and Biopharmaceutics*. 2012; 82: 49–57.
- [38] About HM, Ali AA, EL Menshawe SF, Elbary AA, Nanotransfersomes of carvedilol for intranasal delivery: formulation, characterization and in vivo evaluation, *Drug Deliv*. 2016; 23: 2471-81.
- [39] Tian.N, Thrasher.KD, Gundy.PD, Hughson.MD, Manning.RD, Jr, Antioxidant treatment prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension, *Hypertension*. 2005; 45: 934- 9.
- [40] Cowley.AW, Jr., Stoll.M, Greene.AS, Kaldunski.ML, Roman.RJ, Tonellato.PJ, Schork.NJ, Dumas.P, and Jacob.HJ, Genetically defined risk of salt sensitivity in an intercross of Brown Norway and Dahl S rats, *Physiol Genomics*. 2000; 2: 107-15.
- [41] Francesc Torrades, SamuelSaizJosé, AntonioGarcía-Hortalb, Using central composite experimental design to optimize the degradation of black liquor by Fenton reagent. *Desalination*. 2011; 268: 97-102.