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Original Article

Fabrication and Evaluation of Controlled Release Transdermal Drug Delivery System of Carvedilol using Design Expert® Software for the Management of Hypertension

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ABSTRACT

Objectives: Design, development and characterization of controlled release transdermal drug delivery system of carvedilol using Design Expert® software for managing hypertension. **Materials and Methods:** CV undergoes extensive first-pass metabolism due to low oral bioavailability approx ~24% and biological half-life ~6 hr. Available CV preparations have drawback of poor patient compliance due to increase in dosing frequency and making the therapy less effective. Phospholipid E80, glycerol and cholesterol were used for preparing CVGs via film hydration technique. Optimization was done using Central Composite Design under Design Expert software. While PVP and EC in a ratio of 4:1 in chloroform (5 mL) with plasticizer dibutyl phthalate (30%) were used for preparing Matrix type transdermal patch containing CVGs. **Results:** Optimized CVGs showed 115.7 nm particle size, and -16.4 mV zeta potential. TEM analysis also showed similar vesicle size and reveals globular structure of CVGs. Nearly 0.31 mm thickness, 0.14 % g weight variation, 99.13% flatness and 98.72% drug content was found in Matrix type transdermal patch having CVGs Transdermal patch with Franz diffusion cell showed approx. 90% release upto 48 hr during *in-vitro* permeation studies. *In-vivo* pharmacological assessment was done for efficacy estimation of GVs transdermal patch by N-nitro-L-arginine methyl ester which produced significant hypertension in rats. The application of CVGs transdermal patch resulted in a gradual decrease in BP, with the maximum effect from the patch observed at 10 hr ($p < 0.001$), the effect continued for 48 hr clearly indicating the gradual release of drug by transdermal patch for a long period. **Conclusion:** Developed novel transdermal patch having CV loaded glycerosomes may be considered as a promising approach for controlled release of drug with effective hypertension management.

Keywords: Transdermal, Carvedilol, Design Expert, Glycerosomes, Hypertension, CCD.

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INTRODUCTION

Nowadays controlled delivery of many drugs have been designed quite frequently by using variety of polymers.[1] Similarly there are various types of transdermal drug delivery systems (TDDS) have also been formulated for releasing drug into systemic circulation at controlled rates.[2] TDDS having matrix approach releases the drug at controlled rate, formed by making drug dispersion in an inert matrix made up of polymer(s). Release of drug from these matrices may be altered by variations in the dimensional patch parameters and polymer matrix material.[3] Carvedilol is the most widely prescribed drug for long-term management of hypertension. The oral administration of carvedilol is rapidly absorbed from gastrointestinal tract (80%), but the oral bioavailability remains low (24%) due to short plasma half-life (approx 6 hr) and significant first-pass metabolism by cytochrome P450 (urinary recovery as unchanged carvedilol is less than 0.3% when compared to oral administration dose).[4] Long term treatment for hypertension using carvedilol often shows poor patient compliance because of short half-life and decreased bioavailability and hence increasing frequency of administration.[5] So, an alternative path for drug administration is required and transdermal route is the best alternative for such drugs. The trasndermal route has many advantages such as bypassing first-pass metabolism, improving patient compliance, controlled and sustained drug release, maintaining steady-state plasma level in particular time interval, minimizing inter- and intra-patient variability, drug therapy may be terminated rapidly by removal of TDDS patches from the skin surface and it could avoid GIT drug absorption difficulties caused by enzymatic activity, gastrointestinal pH, and drug interactions with other orally administered medicines, drink, and food.[6-7] Carvedilol possesses ideal characteristics, like poor oral bioavailability short plasma $t_{1/2}$, a suitable logarithmic partition coefficient (log octanol/water: 0.58 ± 0.02; log octanol/buffer pH 7.4: 0.64 ± 0.07), low molecular weight (406.5), and smaller dose range (25–40 mg) for formulating a transdermal patch.[8]

Extensive literature survey described that different ratios of polyvinyl pyrrolidone (PVP) and ethyl cellulose (EC) can be used for preparing controlled release TDDS.[9-10] PVP added as a hydrophilic component to the patch formulations enhances the rate of drug release when compared to hydrophobic film former such as EC. This can be explained via leaching of soluble components, due to which pores are formed and hence decrease mean diffusion drug molecules path length release into the dissolution medium.[10] Conversely, in polymer matrix type, the solid drug is uniformly dispersed in a lipophilic or hydrophilic polymer matrix

and a drug reservoir is formed, which is then molded into medicated disks.[11] It is designed with controlled thickness and a defined surface area. These disks are mounted on to occlusive base plates to form a drug impermeable backing.

The main objectives of study were to: (1) Design and develop transdermal matrix type patches with different combinations of hydrophobic and hydrophilic polymer, like PVP and EC with drug (carvedilol) and plasticizer (30%), (2) Evaluate the physicochemical characterization like weight variation, thickness, folding endurance, and so on, and (3) Determine the patches efficacy through *in-vitro*, *ex-vivo*, and *in-vivo* studies against hypertension-induced rats. The rationale was to improve bioavailability by delivering drug across intact skin at a controlled rate and managing hypertension for a longer period from optimized transdermal patches.

MATERIALS AND METHODS

Materials

Carvedilol was provided as gift sample by the Madras Pharmaceuticals, Chennai. Cholesterol, glycerol, methanol (HPLC grade), acetonitrile (LC/MS grade), and Tween 80 (extra pure grade) were purchased from S.D. Fine Chemicals Pvt. Ltd. (Mumbai, India). All chemicals were used as received without further purification. All other chemicals were of analytical grades. Milli Q HPLC water (Millipore, USA) was used for analysis. The protocol for animal experimentation was permitted by Institutional Animal Ethics Committee (IAEC) of INMAS, DRDO.

Preparation of Placebo Glycerosomes (GVs) and Carvedilol loaded Glycerosomes Vesicles (CVGs)

Although several preparation methods of conventional glycerosomes are given in the literature, film hydration technique was selected in this study because it had been the common and scalable technique resulting in uniform glycerosomes. GVs were formulated by dissolving different amounts of the lipid (50-90 mg) and cholesterol (5-15 mg) in 5.0 ml mixture of chloroform and methanol in a round-bottomed flask (2:1 v/v). Keeping under vacuum, evaporation of organic solvent was done with help of a rotary evaporator (Hahnvapor, Hahnshin S&T Co. Ltd., Korea) at 37°C temperature with 100 rpm rotational speed. To remove traces of solvent, dried film was kept under reduced pressure for two hours. The film was left overnight to dry and then shaken vigorously using aqueous medium (5 mL) containing glycerol to form vesicular dispersion after rehydration by further rotation at 60°C for 1 hr. Nanosized dispersions were acquired by sonicating the formed dispersions by ultrasonicator (Hielscher Ultrasonics GmbH, Germany) at amplitude of 80% for 6 min. Whenever required, CV (2.5 % w/w) was added into the organic phase.[12-13]

Optimization of Carvedilol loaded Glycerosomes Vesicles (CVGs) by using Design Expert

To synthesize the formulation with optimum characteristics, Central composite design (CCD) was chosen and applied using Design Expert® version 11.0.0 by Stat-Ease, Inc. (Suite 480, Minneapolis, MN, USA). Because it generates more runs than other designs in the Design Expert software, the Central Composite Design (CCD) was chosen for the formulation optimization.[14] The formulation was optimized by using central composite design at two levels, i.e. twice, because the formulation required to be loaded with drugs and the necessary properties of the formulation were specific. Each variable was assigned to either a low (-1) or a high (1) value. The software itself created midway value (0) and generated combinations with three different values, -1 (low), 0 (medium), and +1 (high) to meet the rotatability requirement in design.

Table 1: (A) Different levels and independent variables used in Design Expert software.

Independent variables	Levels		
	Low (-1)	Medium (0)	High (+1)
X_1= Conc. of lipid (mg)	50	70	90
X_2= Conc. of glycerol (mg)	10	20	30
X_3= Conc. of cholesterol (mg)	5	10	15

Table 1: (B) Applied constraints on independent and dependent variables.

Independent variables	Constraints	Importance
X_1= Conc. of lipid (mg)	In range	-
X_2= Conc. of glycerol (mg)	In range	-
X_3= Conc. of cholesterol (mg)	In range	-
Dependent variables	**Constraints**	**Importance**
Y_1 = Vesicle size (nm)	Minimize	+++++
Y_2 = Entrapment efficiency (%)	Maximize	+++++
Y_3 = Deformability index	Maximize	++++

In this study, Central composite design has been used as an optimization tool. Concentrations of lipid (mg), glycerol (mg) and cholesterol (mg) were selected as independent variables based on their potential to influence the entrapment efficiency (%), vesicle particle size (nm), and deformability index. The high and low levels of independent variables were selected from rigorous literature survey and previously done screening studies. The medium, -α and +α levels were auto-selected by the software (Table 1A). Depending upon the entered independent variables, CCD had given 17 randomized formulation runs, out of them 3 were center points, 8 runs were factorial points, and 6 were axial points. The constraints were used as per the requirements to attain the best formulation (Table 1B).

The software generated quadratic polynomial equation for the three-factor three-level is given below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

In vitro Characterization

Particle Size, Size Distribution, and Zeta Potential

All prepared formulation was observed for the various parameters. With the help of DLS technique, the mean vesicle size of various formulations were determined and quantify. Poly dispersity index (PDI) was also calculated using Delsa Nano C, Beckman Coulter Counter. For getting better results, diluted suspension of formulations (10 times) with distilled water was prepared and kept in particle size analyzer. The zeta potential is one of the most important indicators of the stability of nanoformulations, thus it was determined using the same approach with the Delsa Nano C Zetasizer.[12,15]

Electron Microscopy

Electron microscopic analysis was used to confirm the size of the glycerosomes and the successful coating on the surface. A drop of sample was put on a copper grid coated with fomvar for transmission electron microscopy (TEM). After that, 2% phosphotungstic acid (one drop) was used to soak the grid for 20 seconds before being allowed to dry. TEM facility was availed from AIIMS, New Delhi. The prepared grid was

Table 2: Design Expert recommended formulations and results of dependent variables obtained (*n*=3).

Formulation code	Independent variables			Dependent variables					
	Coded factors			Obtained value			Predicted value		
	X1	X2	X3	Y1	Y2	Y3	Y1	Y2	Y3
GV 1	+1	-1	0	162.7	80.5	21.8	164.98	79.92	21.66
GV 2	0	0	0	118.4	88.4	26.7	116.92	88.42	26.46
GV 3	0	0	0	118.5	88.7	26.2	116.92	88.42	26.46
GV 4	0	0	0	116.3	88.1	26.4	116.92	88.42	26.46
GV 5	-1	0	-1	135.5	78.1	20.7	135.75	77.14	20.96
GV 6	0	-1	+1	132.7	82.6	22.8	130.68	82.21	23.20
GV 7	+1	+1	0	165.1	79.6	21.7	163.43	79.25	21.59
GV 8	0	0	0	117.2	87.6	25.5	116.92	88.42	26.46
GV 9	+1	0	+1	166.7	75.9	22.3	166.45	76.86	22.04
GV 10	0	-1	-1	139.3	78.5	20.9	137.38	79.11	20.52
GV 11	-1	-1	0	134.8	81.2	22.4	136.48	81.55	22.51
GV 12	+1	0	-1	171.8	76.8	19.3	171.45	76.76	19.81
GV 13	0	0	0	114.2	89.3	27.5	116.92	88.42	26.46
GV 14	0	+1	+1	124.5	81.3	22.5	126.43	80.69	22.88
GV 15	0	+1	-1	124.9	79.8	21.6	126.93	80.19	21.20
GV 16	-1	+1	0	125.6	81.2	22.8	123.33	81.77	22.94
GV 17	-1	0	+1	133.2	80.6	23.6	133.55	80.64	23.09

Table 3: Regression analysis (ANOVA) results for Y^1 (Vesicle Size nm), Y^2 (% EE), Y^3 (DI).

Quadratic model	R^2	Adjusted R^2	Predicted R^2	SD	% CV
Response (Y_1)	0.9926	0.9831	0.9116	2.52	1.86
Response (Y_2)	0.9827	0.9606	0.7985	0.88	1.07
Response (Y_3)	0.9621	0.9133	0.7326	0.70	3.03

analyzed by TEM (Philips CM 10, Holland) at an accelerating voltage of 100kv. Data acquisition was done on the AMT Image Capture Engine.[15]

Differential Scanning Calorimetry (DSC)

DSC is commonly used to assess the thermal behaviour of lipid bilayers, and any interaction between an external substance and the bilayers is seen as a change in lipid Tm. Increase in Tm of lipid due to addition of exogenous substance indicates rigidity of bilayer whereas reduction in Tm of lipid is indicative of fluidity.

For thermal analysis, 3-5 mg of the sample was put in the DSC pan and compressed. The thermogram of the carvedilol loaded glycerosomes and pure lipid were then obtained in the temperature range 10°C-300°C at a heating rate of 10°C/min under an inert atmosphere flushed with nitrogen gas at 20mL/min flow rate using Perkin Elmer, USA, Pyris 6-DSC. Thermograms of DSC were analyzed by Pyris Thermal Analysis software (v4.01).[16]

Preparation of Matrix Type Transdermal Patch

The solvent evaporation technique was used to prepare Matrix-type transdermal patches in a Petri dish containing aluminum foil as backing membrane. Solution of ethyl cellulose (EC) and polyvinyl pyrrolidone (PVP) in a ratio of 1:4 were prepared in chloroform (5 mL). Optimized GVs containing carvedilol (2.5% w/w) was added along with plasticizer dibutyl phthalate (30%) to the polymeric solution. Petri dish was used to pour the solution on aluminium foil and allowed to air-dry overnight. Circular patches of 2 cm diameter (3.14 cm^2) were cut from semi-dried patches and placed in desiccators until further use.[17]

Evaluation of Physicochemical Parameters of Prepared Transdermal Patch

Thickness: Patch thickness was measured using digital vernier caliper at 3 different sites of each patches and the mean was taken.[18-19]

Weight Variation: For weight variation analysis randomly three patches were selected and weighed individually for each formulation and the average was taken.[18-19]

Flatness: Each patch was sliced into three longitudinal strips: one from the left side, one from the centre, and one from the right side. Each strip's length was measured, and the difference in length due to non-uniformity in flatness was calculated using percent constriction, with 0 percent constriction equaling 100 percent flatness.[18-19]

$$\text{Construction}\,(\%) = (L_1 \cdot L_2) \times 100$$

Where, L_1 is each strip's initial length and L_2 is the final length.

Drug Content: One 3.14 cm^2 patch was immersed in 100 mL phosphate-buffered saline (pH 7.4) and continuously shaken vigorously for 24 hours. For 15 min, the entire solution was ultrasonicated. Following filtering, the drug's content was evaluated by spectrometric analysis at a wavelength of 240 nm.[18-19]

In vitro Skin Permeation Studies

A Franz diffusion cell (JFDC 07, Orchid Scientific) was used to performed *in-vitro* drug release studies with 7 ml capacity of receptor compartment. The drug content of matrix-type transdermal patches was determined using a cellophane membrane having pore size 0.45 µm was mounted between the receptor and donor compartment of a diffusion cell. The prepared transdermal matrix type patch was kept on the cellophane membrane. The drug molecule passes through cellophane

membrane easily from patches like rat skin, so cellophane membrane was chosen as standard membrane for *in-vitro* study. The diffusion cell's receptor compartment was filled with phosphate-buffered saline (pH 7.4). The entire assembly was mounted on a three-station diffusion cell apparatus, and the solution was constantly agitated with magnetic beads at 600 rpm in the receptor compartment while maintaining a temperature of 32°C ± 0.5°C. The samples were taken at various intervals and spectrophotometrically evaluated for drug concentration at 240 nm. At each sampling interval, the receptor phase was replenished with an equal volume of phosphate-buffered saline.[19-20]

In vivo Pharmacological Study

A blood pressure (BP) monitoring apparatus (Wood Dale, Stoelting, IL) with digital BP display panel and a noninvasive tail cuff method was used. The rats were taught and trained to remain in the rat cage in a calm and nonaggressive state during BP measurement. After recording the initial BPs, LM (20 mg/kg/ week subcutaneously) was injected for inducing hypertension. Rats with a minimum mean blood pressure of 150 mm Hg were chosen two weeks later. Three groups of animals ($n = 6$) were formed. Group I acted as the control group, while groups II and III got carvedilol (5 mg/kg orally) and GVs transdermal patch, respectively. At various time intervals, blood pressure was measured (1, 2, 4, 6, 10, 24, 36 and 48 hr).[17]

Statistical Analysis

The $p < 0.05$ was considered to be significant. All the collected and quantified data were analyzed statistically using one-way and two-way ANOVA by Tukey's test and Benforini test respectively. All the results were showed as mean ± standard deviation (SD).

RESULTS

Preparation and Optimization of Carvedilol Loaded GVs Using Design Expert

GVs were formulated using film hydration technique and optimized using Design Expert® version 11.0.0 by Stat-Ease, Inc. (Suite 480, Minneapolis, MN, USA).

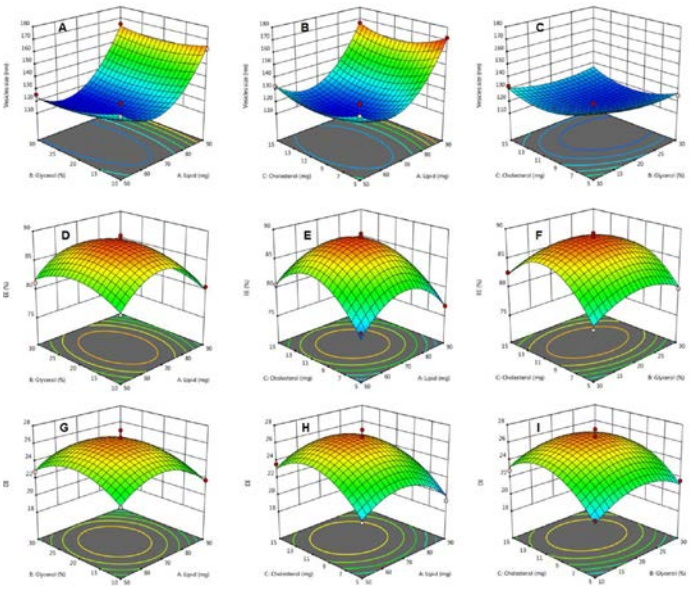

Figure 1: 3-D response surface plots showing the interaction effect for vesicle size, EE% and DI as a function of Concentration of lipid, glycerol and cholesterol.

Response Analysis

Vesicle Size: The particle size of nanoformulation is a critical characteristic since it influences the formulation's performance, targeting ability, and fate in the body. Furthermore, because drugs would eventually be loaded into the glycerosomes, the size of the glycerosomes had to be kept to a minimum from beginning. The R^2 value (correlation coefficient) of 0.9926 indicated that the model was well-fitting as it approached. Furthermore, the difference between the Predicted R^2 of 0.9116 and the adjusted R^2 of 0.9831 is less than 0.1. The effect of independent variables on the size of the final glycerosomes is represented by Equation (1).

$$Y_1 = +116.92 + 17.15\,X_1 - 3.68\,X_2 - 1.80\,X_3 + 2.90\,X_1X_2 - 0.70\,X_1X_3 + 1.55\,X_2X_3 + 25.79\,X_1^2 + 4.34\,X_2^2 + 9.09\,X_3^2 \quad (1)$$

The average size was in range of 26.568 nm to 114.202 nm. According to the above equation, lipid concentration has the greatest impact on glycerosome size, followed by glycerol concentration, while cholesterol concentration has the least impact. Size of the vesicles increases with rise in lipid concentration. Positive effects of linear terms of lipid content have been observed on size, whereas glycerol has a negative effect. As a result, the size of glycerosomes grows as lipid concentration increases and decreases as glycerol concentration increases (Figure 1).

Entrapment Efficiency: To make formulation and the entire process more cost effective, the drug's entrapment efficiency should be constrained to the utmost extent possible. Equation 2 depicts the effect of independent factors on glycerosome entrapment efficiency.

$$Y_2 = +88.42 - 1.04\,X_1 - 0.1125\,X_2 + 0.90\,X_3 - 0.2250\,X_1X_2 - 0.850\,X_1X_3 - 0.650\,X_2X_3 - 5.25\,X_1^2 - 2.55\,X_2^2 - 5.32\,X_3^2 \quad (2)$$

Entrapment efficiency values ranged from 75.9% to 89.37%. The linear terms of lipid and glycerol concentrations have a negative impact on entrapment efficiency, while the linear term of cholesterol has a positive impact. As shown by equation 2, cholesterol concentration has the greatest impact on entrapment efficiency, followed by glycerol and lipid concentration. As the lipid content rises, more and larger glycerosomes form, resulting in better entrapment efficiency. Increased glycerol concentration, on the other hand, results in smaller glycerosomes, which means less drug entrapment.

Deformability Index: The lipid content's linear term has a negative effect on Deformability Index whereas linear terms of cholesterol and glycerol have positive effects. As shown in equation 3, Deformability Index is most affected by cholesterol concentration and then glycerol, followed by lipid concentration less effectively.

$$Y_3 = +26.46 - 0.550\,X_1 + 0.0875\,X_2 + 1.09\,X_3 - 0.1250\,X_1X_2 + 0.0250\,X_1X_3 - 0.250\,X_2X_3 - 2.38\,X_1^2 - 1.91\,X_2^2 - 2.60\,X_3^2 \quad (3)$$

Based on three quadratic equations for the dependent variables and constraints applied on them, CCD predicted the optimized GVs.

According to CCD, the final recipe for optimized GVs was comprised of 65.993 mg lipid, 20.681 mg glycerol and 10.653 mg of cholesterol. The Vesicle size in nm, EE% and Deformability index of this optimized GVs were predicted as 114.202 nm, 88.44 % and 26.568 respectively with the solution desirability of 0.944. The theoretically optimized GVs were translated experimentally and six formulations were formed for the validation of vesicle size in nm, EE% and Deformability index (Table 4).

In vitro Characterization

Particle Size and Size Distribution: The vesicle size of optimized nanoformulation was analyzed by Malvern Zeta-sizer and was measured 115.7 ± 6.31 nm with PDI of 0.180 (Figure 2).

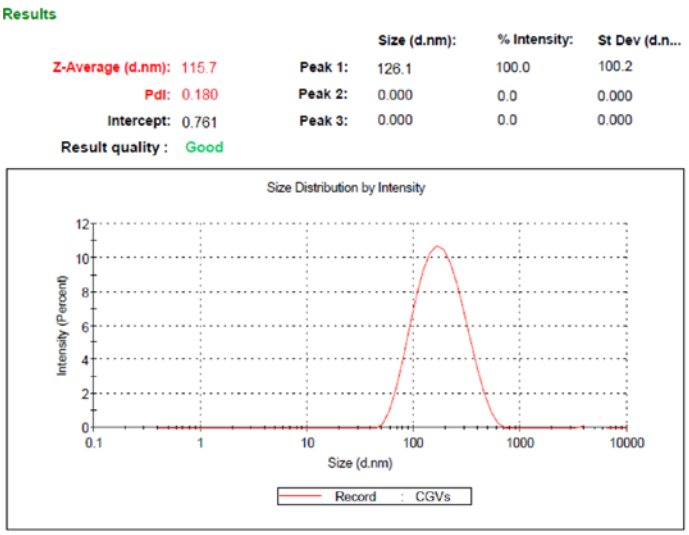

Figure 2: Particle Size distribution of Carvedilol loaded glycerosomes.

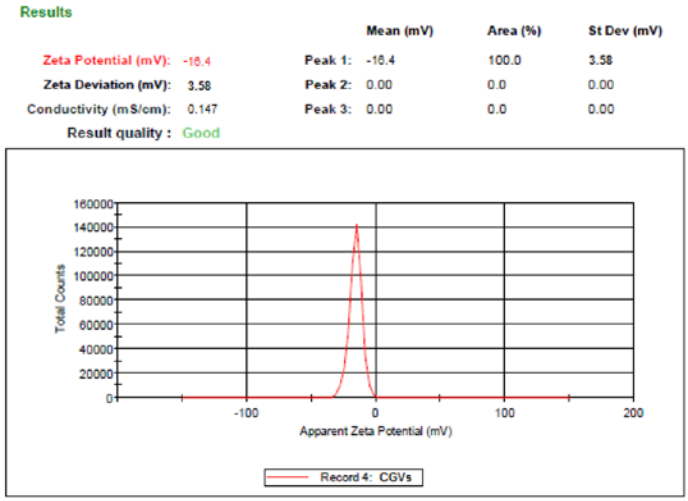

Figure 3: Zeta Potential of Carvedilol loaded glycerosomes.

Table 4: Optimal values of independent variables and experimentally determined dependent variables for optimized batch of GVs *(n=6)*.

Batch	Independent variables			Dependent variables		
	X_1	X_2	X_3	Y_1	Y_2	Y_3
Predicted	65.993	20.681	10.653	114.202	88.444	26.568
Experimental	65.993	20.681	10.653	115.7 ± 13.1	87.3 ± 8.5	27.2 ± 4.9

Zeta Potential: The zeta potential of optimized nanoformulation was analyzed by Malvern Zeta-sizer and was measured – 16.4 mV (Figure 3).

Electron Microscopy: TEM image (figure 4), showed somewhat regular and smaller structure of glycerosomes. The results of the TEM examination agreed with those of the zeta-sizer particle size analysis. Also, there were no signs of liposomal structures aggregation revealed by TEM analysis.

Differential Scanning Microscopy: Figure 5 showed a pure lipid thermotropic transition at 41°C. GV formulation made up of lipid and cholesterol shows a thermotropic transition at 45°C. The rise in Tm of lipid could possibly be because of the presence of cholesterol in

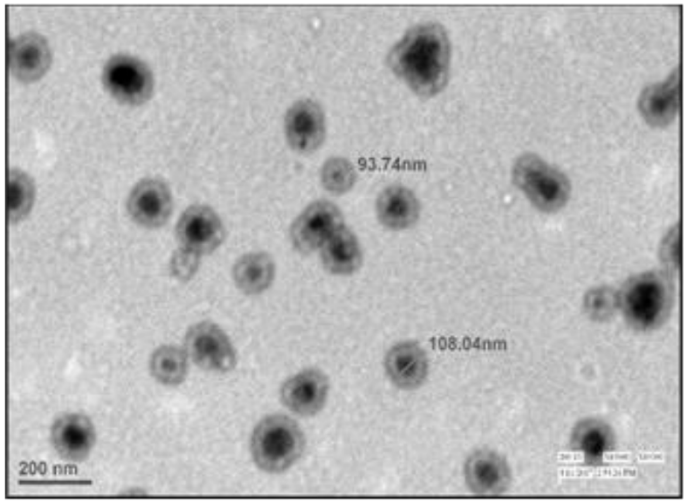

Figure 4: TEM image of Carvedilol loaded glycerosomes.

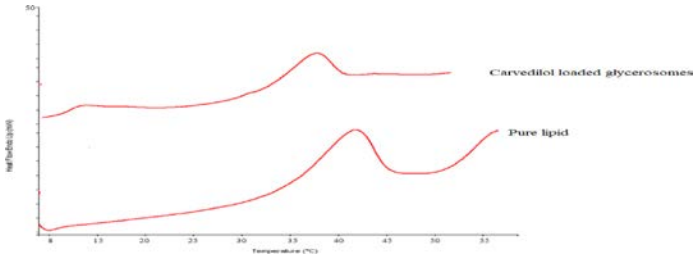

Figure 5: DSC image of pure lipid and Carvedilol loaded glycerosomes.

the nanoformulation. The existence of cholesterol applied a profound but complicated influence on lipid bilayer properties due to its unique physical characteristics. Despite cholesterol considered as lipid, it has less resemblance to phospholipids. Cholesterol's hydrophilic domain is very small, consisting of only one hydroxyl group. A hard planar structure formed of many fused rings is adjacent to this hydroxyl group. A single chain tail extends from the other end of the ring structure. For decades, scientists have known that adding cholesterol to a fluid phase bilayer reduces its flexibility. This interaction also boosts the mechanical rigidity and lowers the lateral diffusion coefficient of fluid membrane lipid bilayers.[21]

Preparation and Evaluation of Prepared Transdermal Patch

Different batches of matrix type transdermal patches loaded with GVs using solvent evaporation technique were prepared as explained in methods section. Circular patches of 2 cm diameter (3.14 cm^2) were cut from semi-dried patches and evaluated as per their physicochemical parameters. The evaluation tests results were summarized in the Table 5.

Thickness confirmed that the patches prepared were uniform in thickness. The incorporation at 30% w/w concentration of dibutyl phthalate of dry polymer weights yielded flexible and smooth patches. The results of the weight variation test showed that the weights of several batches of patches were substantially similar. The drug content was found to be consistent across batches. Formulation had the same strip length before and after their cuts, according to the % flatness study. As a result, there was no constriction; all patches had a flat and smooth surface, which could be maintained when placed to the skin.[22]

In vitro Skin Permeation Studies

Franz diffusion cell was used for carrying out *in-vitro* skin permeation studies and results are depicted in Figure 6. It was clearly shown that the CVGs patch exhibited (91.41 ± 7.39) percentage drug release values, which was significantly ($p < 0.05$) different compared to plain GVs (58.63 ± 4.71). Different kinetics (zero-order, first-order, Peppas, Higuchi kinetics, and Hixson–Crowell) models have been tried to describe the dissolution profile by a model function. Higuchi kinetics model considered as the most appropriate model describing release kinetics from the matrix type prepared transdermal patch ($R^2 = 0.994$), which indicates that the drug permeation was governed by a diffusion-dominated mechanism.[23]

In vivo Pharmacological Study

Table 6 showed that the injection of N-nitro-Larginine methyl ester (NLME) to rats resulted in severe hypertension. The administration of carvedilol orally controlled hypertension strongly ($p < 0.001$) at first, with the largest effect noted at 2 hr, however after 2 hr, the BP steadily climbed until it was virtually the same as the starting value after 24 hours. The administration of CVGs transdermal patch, on the other hand, led in a steady decrease in BP, with the patch's highest effect occurring at 10 hr ($p < 0.001$), and the effect lasting for 48 hr, indicating that the transdermal patch releases the medication gradually over time.

Initially, there was no significant difference in BP between the patch and the oral group ($p < 0.05$) until the first 2 hr, however the impact of oral carvedilol began to fade after 6 hr due to its short half-life. The CVGs transdermal patch was able to manage hypertension for the entire 48-hr period because it provided continuous and sustained drug delivery. Clearly, the patch might overcome the disadvantages of carvedilol administration by mouth, such as short half-life, low bioavailability, and rapid first-pass metabolism.

Table 5: Physicochemical Properties of GVs loaded prepared transdermal patches.

Parameters	GVs Transdermal Patch
Thickness (mm)	0.31 ± 0.02
Weight variation (%) (g)	0.14 ± 0.01
Flatness (%)	99.13 ± 0.7
Drug Content (%)	98.72 ± 0.5

All data expressed as mean ± SD (n=3)

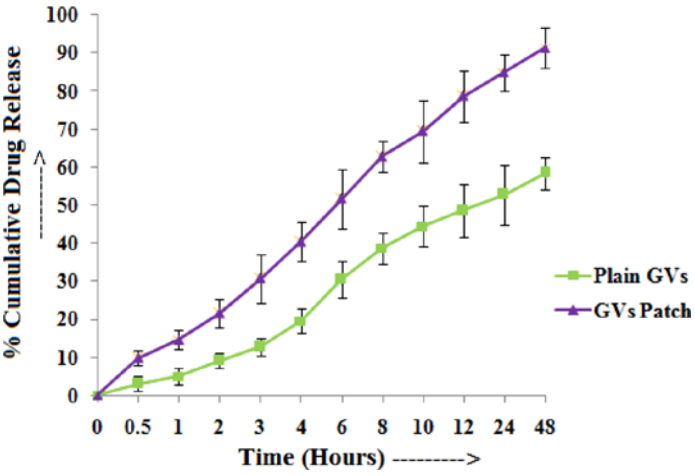

Figure 6: *In-vitro* permeation graph of transdermal patch containing carvedilol-loaded glycerosome vesicles. Data shows mean ± standard deviation ($n = 3$).

DISCUSSION

This article explains the formulation of carvedilol loaded glycerosomes using modified film hydration technique and then optimization was done using CCD. Concentrations of lipid (mg), glycerol (mg) and cholesterol (mg) were chosen as independent variables and the entrapment efficiency (%), the vesicle particle size (nm), and deformability index were chosen as dependent variables (Table 1A and 1B). Design Expert recommended 17 formulations and experimentally determined values of dependent variables obtained were given in Table 2. According to constraints applied and fed experimentally data in the software, CCD predicted the optimized CVGs and optimal value of independent and dependent variables for optimized batch (Table 4) with the desirability of 0.944.

The optimized CVGs were evaluated for vesicle size, zeta potential, TEM, and DSC analysis. The vesicle size and zeta potential were found to be 115.7 ± 6.31 nm with PDI of 0.180 (Figure 2) and be – 16.4 mV (Figure 3) respectively. TEM analysis showed regular and smaller structure of glycerosomes which also indicated that liposomal structures were devoid of aggregation (Figure 4).

The solvent evaporation technique was used to make matrix type transdermal patches loaded with CVGs and evaluated on physicochemical parameters (Table 5). The addition of dibutyl phthalate yielded smooth and flexible patches which could be applied to the all types of skin easily.[22]

Franz diffusion cell was used to carry out *in-vitro* drug release studies (Figure 6). CVGs patch showed much higher drug release than plain GVs which may be due to the dissolution of aqueous soluble fraction of the formulation, which leads to pores formation, and hence drug molecules released into dissolution medium due to decrease mean diffusion path length and led to higher release rates.[23]

Finally, the *in-vivo* pharmacological study was performed on 18 rats divided in 3 groups. For inducing hypertension in rats N-nitro-Larginine methyl ester (NLME) was used. The results were summarized in Table 6 which showed that the application of CVGs transdermal patch caused a progressive decrease in BP upto 48 hr showing its ability for continued and sustained drug release. Throughout the duration, the patch was able

Table 6: Antihypertensive effect of prepared transdermal patch in comparison to oral route.

Gr.	Treatment	Initial	1 h	2 h	4 h	6 h	10 h	24 h	36 h	48 h
1	Control	179.61 ± 8.19	177.18 ± 5.71	177.63 ± 7.53	179.29 ± 5.61	179.35 ± 8.24	178.43 ± 7.49	178.72 ± 6.33	179.11 ± 7.29	178.05 ± 7.15
2	CV Oral	178.83± 7.35	127.49 ± 6.11[a]	90.75 ±6.21[a]	96.27 ±7.38[a]	104.62 ± 5.74[a]	115.89 ± 6.31[a]	171.41 ± 7.25	174.39 ± 5.81	175.12 ± 5.72
3	GVs Transdermal Patch	179.59 ± 7.28	152.46 ±6.80[a,b]	137.28 ±6.51[a,b]	114.91 ±7.32[a,c]	109.69 ±7.11[a,c]	99.35 ±6.74[a,c]	102.51 ±5.17[a,b]	106.76 ±6.41[a,b]	113.49 ±6.16[a,b]

[a]Significant compared with control ($p < 0.001$); [b]Significant compared with oral ($p < 0.001$); [c]Not significant compared with oral ($p > 0.05$). All data expressed as mean ± SD ($n = 6$).

to keep the hypertension under control and used as standalone therapy for overcoming the disadvantages of available oral carvedilol systems such as short half-life, high first-pass metabolism, and low bioavailability.

CONCLUSION

In this project work, the carvedilol loaded glycerosomes were successfully developed and optimized using QbD. For getting optimized GVs, Central Composite Design anticipated the independent variables and their solutions based on constraints. The observed and predicted values for dependent variables were highly correlated. The vesicle size of optimized GVs determined by DLS was further authenticated by TEM analysis. TEM analysis also confirmed the globular structure of optimized GVs. Transdermal matrix type patch containing CV loaded Glycerosomes were successfully prepared and evaluated. The developed patch was proven to be extremely useful and is a feasible choice for controlling and effectively managing hypertension. EC in combination with PVP has a good patch-forming property. Incorporation of 30% w/w dibutyl phthalate resulted in smooth, flexible, and uniform patches. Also, pharmacodynamic study found that the developed patch decreased the BP gradually and significantly ($p < 0.001$), that is, prolonged control of hypertension for 2 days. Results of pharmacodynamic studies revealed that the prepared patch was able of surmounting the effect of carvedilol when taken orally and used as standalone therapy in controlling and managing hypertension.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BBD: Box Benken Design; **CCD:** Central Composite Design; **CV:** Carvedilol; **CVGs:** Carvedilol Loaded Glycerosomes; **DI:** Deformability Index; **DLS:** Dynamic Light Scattering; ***DSC:*** *Differential Scanning Calorimetry;* **EC:** Ethyl Cellulose; **EE:** Entrapment Efficiency; **GVs:** Glycerosomes Vesicles; **HPLC:** High Performance Liquid Chromatography; **MCT:** Medium Chain Triglyceride; **NLME:** N-nitro-L-arginine Methyl Ester; **PDI:** Poly Dispersity Index; **PVP:** Polyvinyl Pyrrolidone; **SD:** Standard Deviation; **TDDS:** Transdermal Drug Delivery System; **TEM:** Transmission Electron Microscopy.

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