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Development and Characterization of A Nanoemulsion Gel formulation for Transdermal delivery of Carvedilol

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Abstract

The aim of the present study was to develop nanoemulsion formulation for transdermal delivery of carvedilol to enhance the water solubility as well as bioavailability of drug. O/W nanoemulsions were prepared by the spontaneous emulsification method. Pseudoternary phase diagrams were constructed to obtain the nanoemulsion region. Oleic acid and IPM (3:1) was chosen as the oil phase, Tween 20 and carbitol were used as surfactant and cosurfactant respectively, on the basis of solubility studies, in formulation of nanoemulsion. Parameters evaluated included: thermodynamic stability testing, droplet size and in vitro mice skin permeation ware performed. Significant difference in the steady state flux (Jss), permeability coefficient (Kp) and enhancement ratio (Er) was observed in nanoemulsion formulations and control (P*** < 0.001). The composition of optimized formulation NEB1 which shows highest value of flux 211.8123 µg $cm^{-2}h^{-1}$ at the 24 h. Post application plasma carvedilol was increased 6.41 fold to marketed dosage form. The study suggested that nanoemulsion significantly enhanced bioavailability of transdermally applied carvedilol and eliminated the first pass metabolism.

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INTRODUCTION

Carvedilol is a non-selective beta blocker indicated in the treatment of mild to moderate congestive heart failure, and it is listed in class II of biopharmaceutical classification of drugs. Carvedilol is a poorly water-

soluble and highly permeable drug [1]. Oral administration of carvedilol is rapidly absorbed from the gastrointestinal tract (80%), but the oral bioavailability remains low (23%) because of significant first-pass hepatic metabolism by cytochrome P450 (urinary recovery as unchanged carvedilol is less than 0.3% of the oral administered dose) [2] . Carvedilol also has a short plasma half-life of 7-10 h [3]. Potential advantages of transdermal /dermal drug delivery (e.g., minimal first-pass metabolism, patient comfort/compliance, local drug delivery to the skin) various physical and chemical approaches have been used to overcome the limiting barrier of drug penetration into the skin [4].

The use of a microemulsion or nanoemulsion as vehicle may enhance transdermal penetration by various mechanism, many molecules or solubilised in microemulsion in addition microemulsion induce a change in the thermodynamic activity of the drug they contain, modifying their partition coefficient and thus favour penetration of the stratum corneum. Furthermore, their component surfactant reduces the functional barrier of stratum corneum. This latter function may be more or less important depending on the nature of the surfactant used [5-6]. Macroemulsions are dispersions of at least two non-miscible liquids. They are thermodynamically unstable systems that are stabilized kinetically [7].

Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size of less than 100 nm[8,9] .

2. MATERIALS AND METHODS

2.1 Materials

Carvedilol was received as gift sample from Cipla Ltd. Mumbai. Oleic acid, isopropyl myristate (IPM), olive oil, triacetin, eucalyptus oil, silicon oil, castor oil. Carbopol-934 was as gift sample from Colorcon India Ltd., Goa. Captex-300, caprol micro express capmul MCM C8, capmul MCM PG8, capmul MCM C8EP ware received as gift sample from ABITEC Corporation, Janesville, WI. Tween-20, Tween-80, Span-80, and carbitol, PEG-200, PEG-400 PEG-600 and propylene glycol ware obtained from CDH, New Delhi. All other chemicals were of analytical grade.

2.2 Solubility Studies

The solubility of drug was determined in different oils viz. oleic acid, isopropyl myristate (IPM), olive oil, triacetin, eucalyptus oil, silicon oil, castor oil, captex-300, caprol micro express capmul MCM C8, capmul MCM PG8, capmul MCM C8EP, surfactant including Tween-20, Tween-80, Span-80,and cosurfactant viz. carbitol, PEG-200, PEG-400 PEG-600 and propylene glycol. 2 ml of different oils was taken in small vials and excess amount of the drug was added. The vials were tightly stopper and were continuously stirred on isothermal water bath shaker for 72 h at 37±0.5°C and then samples were centrifuged at 3000 rpm for 15 min. The supernatant was separated, filtered and after appropriate dilution with methanol, solubility was determined by UV spectrophotometer. Same method was adopted for solubility determination of drug in surfactant and co-surfactant [10].

2.3 Determination of partition coefficient of drug

The partition coefficient of the drug was determined in n-octanol/distilled. 20 mg of drug was accurately weighed and added to a mixture containing 10 ml each of n-octanol and distilled water. The flask was than shaken at 37 ± 0.5 °C for 24 h. The mixture was then transferred to a separating funnel and allowed to equilibrate for 10 h. The aqueous and n-octanol phases were separated and filtered through membrane filter (0.45 µm) concentration of drug was determined by UV Spectrophotometer and partition coefficient was calculated.

2.4 Pseudoternary phase diagram studies

A titration method was employed to construct pseudo-ternary phase diagram. The mixture of oleic acid and IPM (3:1), Tween-20 and Carbitol were selected as oil phase, surfactant and cosurfactant respectively. Distilled water was used as an aqueous phase. For each phase diagram, oil and S_{mix} at a specific ratio was mixed thoroughly at different mass ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and S_{mix}, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.3, 1:2, 1:1.5, 1:1, 1:0.7, 1:0.43, 1:0.25, 9:1 were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Slow with aqueous phase was performed for each mass ratio of oil and S_{mix} and visual observations were made for transparent and easily flow able o/w nanoemulsions. The physical state of the nanoemulsion was marked on a pseudo-three-component phase diagram with one axis representing the aqueous phase, the second one representing oil and the third representing a mixture of surfactant and co surfactant at a fixed mass ratio. The phase boundary was determined in triplicate with accuracy better than ± 2 % (w/w) oil for each system [11].

2.5. Selection of placebo nanoemulsion formulations

From each phase diagram constructed, different formulations were selected from the nanoemulsion region so that carvedilol could be incorporated into the oil phase. Therefore, the following criteria were used for the selection of different formulations from the phase diagrams.

- 6 mg. of carvedilol was selected as a dose for incorporation into the oil phase.
- For convenience, 2 ml was selected as the nanoemulsion formulation, so that it could be increased or decreased as per the requirement in the proportions.
- The oil concentration should be such that it solubilizes the drug (single dose) completely depending on the solubility of the drug in the oil.
- From each phase diagram, different concentrations of oil were selected at a difference of 5% (5%, 10%, 15%, 20%, 25%, etc) from the nanoemulsion region.
- The effect of carvedilol on the phase behavior and nanoemulsion area of the phase diagram was checked.
- For each percentage of oil selected, the formula that used the minimum concentration of S_{mix} for its nanoemulsion formation was selected from the phase diagram.

2.6. Formulation of drug loaded nanoemulsions formulation

For the preparation of drug loaded nanoemulsions, 6 mg of carvedilol was dissolved in the mixture of oleic acid and IPM (3:1). The required amount of S_{mix} was added, after that water was added drop wise till a clear and transparent liquid was obtained on ultra sonication, the composition of nanoemulsion formulations was shown in the table 3.

2.7. Thermodynamic stability studies of carvedilol loaded nanoemulsions formulation

To overcome the problem of metastable formulation, thermodynamic stability tests were performed. Selected formulations were centrifuged at 3500 rpm for 30 min. Those formulations that did not show any phase separations were taken for the heating and cooling cycle. Six cycles between refrigerator temperatures of 4°C and 45°C for 48 h were done. The formulations that were stable at these temperatures were subjected to the freeze-thaw cycle test. Three freeze-thaw cycles were done for the formulations between −21°C and +25°C. Those formulations that survived thermodynamic stability tests were selected for the further studies.

2.8. Characterization of optimized nanoemulsion

The optimized nanoemulsion formulation was characterized for various attributes.

2.8.1. Droplet size and size distribution

Droplet size was determined by photon correlation spectroscopy (PCS) that analyzes the fluctuations in light scattering due to Brownian motion of the droplets using a Zetasizer (1000 HS, Malvern Instruments). The formulation (0.1 ml) was dispersed in 50 ml of water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering was monitored at 25 °C a 90 ° angle [12].

2.8.2. Viscosity

The viscosity of the formulations was determined by using Brokfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratory., Middlebro, MA) using spindle # CPE40 at 25 ±0.5°C. The software used for the calculations was done by Rheocalc V2.6.

2.8.3. Refractive Index

Refractive Index was determined using Abbe's refractometer at 25°C.

2.8.4. pH

The pH of optimized carvedilol loaded nanoemulsion was determined using a digital pH meter.

2.8.4. Conductivity

Conductivity of optimized nanoemulsion formulation was measured using conductometer and current flow was observed.

2.9. Solubility determination

Excess amount of carvedilol was added to 1 ml of the nanoemulsion NEB1 and was shaken at 37±0.5℃ in a water bath shaker for 72 h. After 72 h nanoemulsion was centrifuged at 3000 rpm for 15 min. The supernatant was filtered using 0.45 µm filter membrane. An aliquot amount was diluted with methanol and analyzed by U.V spectrophotometer.

2.10 Statistical Analysis

The data were analyzed statistically by the Repeated Measures Analysis of Variance ANOVA test followed by the least significant difference procedure. This statistical analysis was carried out using GraphPad Prism5.0 Softwares (Inc, San Digeo USA), Graphpad InStat Softwares (Inc, San Digeo, USA). The data from different formulations were compared for statistical significance by Repeated Measures Analysis of variance (ANOVA) (Dunnet multiple comparison test). Differences were considered to be statistically significant when P*< .05, P**< .01. P***< .001, each set of experiments were performed at least three times (n=3).

3. RESULTS AND DISCUSSION

The drug sample was characterized for its authenticity using its monograph. The drug sample was observed for nature, odor, and melting point and solubility. The drug was identified by FTIR & UV. The drug exhibited absorbance maximum at 332 nm, 286 nm, 243 and 224 with maximum absorbance 243 nm which was same as the reported one **(Clark's 2005).**

The FT-IR spectra of the carvedilol confirmed characteristic absorption bands of hydroxyl group at 3346 cm^{-1}, secondary amine 3325 cm^{-1}, unsaturation at 1630, 1607 & 1590 cm^{-1} and aromatic ring at 1502, 956 & 915 cm^{-1}. On the basis of these studies, it was proved that carvedilol was authentic.

3.1 Solubility studies

The solubility of the drug in oils is most important, as the ability of the nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the oil phase. If drug solubilization is due to the surfactant or cosurfactant, there could be a risk of precipitation, particularly in case of oral or parenteral nanoemulsion [13]. After screening the oils for carvedilol solubility, it was found that carvedilol exhibited maximum solubility in the mixture of oleic acid and IPM (3:1). Hence oleic acid and IPM (3:1) was chosen as the oil phase. It has also been reported that oleic acid is a powerful enhancer for transdermal delivery[14] , as it increases the fluidity of the intercellular lipid barriers in the stratum corneum by forming separate domains

which interfere with the continuity of the multilamellar stratum corneum and induce highly permeable pathways in the stratum corneum [15,16] . The solubility of carvedilol in oils, surfactant and cosurfactants was shown in the Table.1&2.

Table 1: Solubility of carvedilol in different oils

S. No.	Oils	Solubility (mg/ml)
1.	Oleic acid: IPM (3:1)	197.94±10.75
2.	Clove oil	161.14±9.91
3.	Oleic acid: IPM(1:1)	148.67±11.35
4.	Oleic acid	116.57±7.25
5.	Oleic acid: Clove oil (1:1)	85.94±9.23
6.	Oleic acid: Clove oil (1:3)	63.99±7.16
7.	Capmul MCMC8	30.26±6.25
8.	Capmul PG8	29.82±4.22
9.	Capro Micro Express	25.14±5.25
10.	Castor oil	16.96±4.29
11.	Captex 355	13.88±3.57
12.	Triacetin	12.11±2.25
13.	IPM	10.65±2.05
14.	Olive oil	9.88±1.85
15.	Eucalyptus oil	4.68±0.95
16.	Silicon oil	0.50±0.15

Table 2: Solubility of carvedilol in different surfactants & cosurfactants

S. No	Surfactants	Solubility (mg/ml)
1.	Tween-20	85.94
2.	Tween-80	29.94
3.	Span-80	49.71
4.	Carbitol	240.45
5.	PEG-200	191.08
6.	PEG-400	233.57
7.	PEG-600	144
8.	Propylene glycol	9.80

The partition coefficient (log P) of carvedilol was found to be 3.5.

Transdermal permeation properties of IPM have also been reported but the mechanism of its action is poorly understood [17].

A crucial point is that surfactant and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region for the desired nanoemulsion type (ie, oil/water, water/oil, or bicontinuous).

The screening of surfactant and cosurfactants on the basis of solubility is difficult because all surfactant and cosurfactant cannot solubilized all type of oil phase. Surfactant chosen must be able to lower the interfacial tension to a very small value to aid the dispersion process during the preparation of the nanoemulsion, provide a flexible film that can readily deform around droplets. On the basis of constructed pseudo ternary phase diagram using carbitol and span-80, carbitol and tween-80, carbitol and tween-20, it was clear that nanoemulsion region in the pseudo ternary phase diagram were increasing in order respectively, because tween-20 had greater oil mixture (Oleic acid: IPM) solubilizing power than tween-80 and span-80 therefore tween-20 were selected as a surfactant for further optimization of cosurfactant.

Cosurfactants are included in the nanoemulsion formulations to provide further reduction in surface tension and to fluidize the interfacial surfactant film. Short and medium chain alcohols were successfully tested as cosurfactants [18], carbitol, PEG- 200, PEG-400, PEG-600, and propylene glycol were selected as cosurfactants in this study. Titrations were done by taking tween-20 as surfactant with different cosurfactant (carbitol, PEG-200, PEG-400 PEG-600 and propylene glycol). A constant surfactant to cosurfactant ratio 1:1 was taken. While oil to S_{mix} ratio was kept 1:9 by keeping in mind that higher concentraction of surfactant or S_{mix} is favourable for maximum nanoemulsion formation. After studying the result, it was found that maximum nanoemulsion region or points were obtained with carbitol and hence carbitol was chosen as cosurfactant for nanoemulsion formulation.

Among the selected surfactant and cosurfactant, pseudo ternary phase diagrams were constructed by aqueous phase titration method in order to define the extent and nature of nanoemulsion region. The

construction of pseudo ternary phase diagrams were started using surfactant Tween-20 and cosurfactant carbitol in different ratios (1:0, 1:1, 1:2, 2:1, 3:1 and 1:3). The existence of nanoemulsion region was highest in 3:1 ratio of Tween-20/carbitol. One interesting result was also seen with this study that with increase of S_{mix} ratio the nanoemulsion region gets increased up the certain limit and vice versa. On the basis of above studies it is concluded that oleic acid, Tween-20 and carbitol was taken as oil phase, surfactant and cosurfactant respectively for further formulation development.

3.2 Preparation of Nanoemulsion Formulations

For the preparation of drug loaded nanoemulsions, required amount of carvedilol was dissolved in the oil phase. The required amount of mixture of surfactant and cosurfactant ware added and double distilled water was then added drop wise drop till a clear and transparent liquid was obtained after ultra-sonication. The prepared nanoemulsions were stored in tightly in the suitable container at ambient temperature. The composition of nanoemulsion formulations were shown in Table 3.

Table 3: Composition of selected nanoemulsion formulation

Formulation Code	Oleic acid + IPM(3:1)(% w/w)	Tween-20 +Carbitol(% w/w)	Surfactant / Cosurfactant (S_{mix})	Distilled Water (% w/w)
NEA1	4.55	40.91	1:1	54.44
NEA2	5.00	45.00	1:1	50.00
NEB1	4.00	36.00	1:2	60.00
NEB2	6.25	40.91	1:2	54.55
NEC1	5.00	45.00	2:1	50.00
NEC2	5.56	50.00	2:1	44.44
NED1	4.00	36.00	3:1	60.00
NED2	4.35	40.91	3:1	54.55

3.3 Droplet size and size distribution

Droplet size was determined by photon correlation spectroscopy (PCS) that analyzes the fluctuations in light scattering due to Brownian motion of the droplets using a Zetasizer (1000 HS, Malvern Instruments). The formulation (0.1 ml) was dispersed in 50 ml of water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering was monitored at 25 °C a 90° angle. The droplet size distribution of the optimized carvedilol loaded nanoemulsion (NEB1) was determined by Malvern Zetasizer (1000 HS, Malvern Instruments, U.K.). The mean droplet size of nanoemulsion (NEB1) was found to be 71.8 nm and was shown in Figure 1.

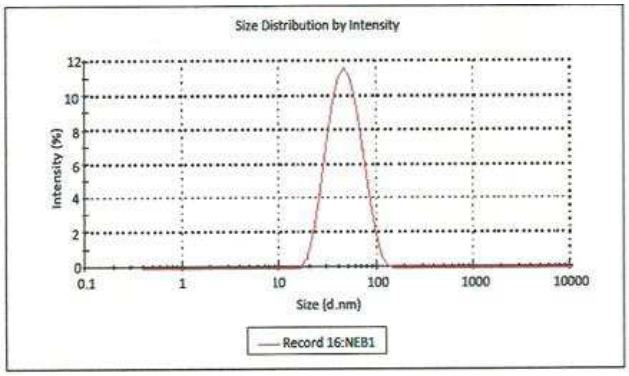

Figure 1: Size distribution of optimized carvedilol loaded nanoemulsion NEB1

3.4 Viscosity

The viscosity of the formulations NEB1 was determined by using Brokfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratory., Middlebro, MA) using spindle # CPE40 at 25 ±0.5°C. The software used for the calculations was done by Rheocalc V2.6 and Viscosity of the nanoemulsion NEB1 formulation was very low as expected for o/w emulsion (29.12 ± 2.05 cP). The low viscosity may be due to presence of low amount of Tween-20 (a fatty acid polyhydric alcohol ester having high intrinsic viscosity) compared to carbitol (a short chain alcohol having low intrinsic viscosity) and the low concentration of oil [19].

3.5 Refractive Index

Refractive Index was determined using Abbe's refractometer at 25°C and the mean value of the refractive index for the formulation NEB1 was found to be 1.417 ± 0.04.

3.6 pH

The pH of optimized carvedilol loaded nanoemulsion (NEB1) was determined using a digital pH meter and was found to be 6.4 ± 0.324 which is favorable for tropical application because the pH of the skin is in the range of 5.5 to 7.0.

3.7 Conductivity

The optimized carvedilol loaded nanoemulsion NEB1 was characterized for conductivity and Conductivity of optimized nanoemulsion formulation (NEB1) was measured using conductometer and current flow was observed.

To overcome the problem of metastable formation, thermodynamic stability tests were performed. The formulations selected were subjected to different stress tests, such as freeze-thaw cycle tests centrifugation heating-cooling cycle, and if the nanoemulsions are stable over these conditions, metastable formulations are thus avoided and frequent tests need not be performed during storage. All the formulations subjected to above studies came back to their original form when subjected to freeze thaw and did not show turbidity or phase separation on high speed centrifugation and thus where found to be stable and suitable for *ex-vivo* permeation study.

For the evaluation of nanoemulsion, various formulations from the phase diagrams with maximum area viz. S/CoS ratio 1:1, 1:2, 2:1, 1:3, 3:1 were selected for in vitro skin permeation studies. In these formulations the content of oil phase was varied from 4.00%-23.26% while the content of surfactant was varied from 36%-54.55%. The effects of the content of oil and surfactant mixture on the skin permeation of carvedilol was evaluated.

3.8 Data analysis

Dose calculation of Carvedilol for TDDS

Transdermal dose = Oral dose × Oral bioavailability in % =25 × 24 = 6 mg

Where A = Css x Cl / Jss

A= absorption area on the skin

Css = steady state conc. in the plasma (17.5 µg/ml)

Cl = total body clearance of carvedilol (36 L/h)

(Therapeutics drugs, 1999 & clacks analysis of drug and poison, 2005)

Jss =drug flux across the skin (211.81 µg/cm²/h)

Thus, the required area of the skin was found to be 2.97 cm².

Thus, assuming an application of 2.97 cm² on human skin would provide the desired steady state conc. in the plasma.

Volume of Franz diffusion cell =5 ml

Area of diffusion cell =0.385 cm²

Drug concentration in donor compartment (C_d) = 6000 µg/ml

The cumulative amount of carvedilol permeated through the skin (Q, µg/cm²) was plotted as function as time (h). The drug flux at steady state (Js, µg/cm²/h) was calculated from the slope of linear portion of the curve.

$$\text{Cumulative amount of drug permeated} = \frac{\text{Concentration (µg/ml)} \times \text{dilution factor}}{\text{Surface area of skin (cm}^2\text{)}}$$

Flux = slope of steady state portion of the plot between cumulative amount of drug permeated vs. time (µg/cm²/h).

Permeability coefficient was calculated by divided by initial drug concentration in donor compartment (µg/ml).

Permeability coefficient (K_p) = Flux/drug concentration in donor compartment (µg/ml).

***3.9 In-vitro* studies**

Approval to carry out *in-vivo* and skin permeation studies was obtained from Institutional Animal Ethics Committee (CPCSEA), Institute of Pharmacy, BU, Jhansi **(Approval no: BU/Pharm/IAEC/09/027)** and their guideline were followed for the studies. On the basis of permeation studies, it was found that nanoemulsion formulation NEB1 consists of 4% oil, 12% tween-20, 24% carbitol and 60% distilled water (from the phase diagram of S/CoS ratio 1:2) exhibited highest carvedilol permeation profile. The cumulative amount of carvedilol permeated from NEB1 was 4901.267µg/cm² at the end of 24h and the skin

permeation rate (flux) of carvedilol was 211.812µg/cm²/h while all other nanoemulsion exhibited lesser percentage of drug permeation and flux.

Table 4: In vitro parameters of carvedilol Nanoemulsion formulation

Formulation Code	Flux (µg/cm²/h)	Permeability Coefficient Kp ×10⁻² (cm/ h)	Enhancement ratio with Control (Er)
Control	2.8641 ±0.03	0.04	----------
NEA1	194.7474 ±23.98 ***	3.24	67.996
NEA2	182.0116 ±25.09***	3.03	63.5493
NEB1	211.8123 ±20.76 ***	3.53	73.9542
NEB2	194.9507 ±21.09 ***	3.23	68.067
NEC1	171.1347 ±24.86 ***	2.85	59.7516
NEC2	165.0647 ±17.23 ***	2.75	57.6323
NED1	196.3196 ±27.54 ***	3.27	68.545
NED2	186.4547 ±27.72 ***	3.10	65.1006
NEB1Gel	190.2591 ±25.58 ***	3.17	66.4289

Values are mean ± SD, n=3, P*** <0.001, As compared to control, Repeated Measures Analysis of Variance ANOVA (Dunnet multiple comparison test)

The content of oil also played an important role in nanoemulsion formulation and it affected the skin permeation rate directly. Oleic acid is advantageous to use as oily phase because it increases skin permeability by two mechanistic scenarios of enhancer; (a) lipid fluidization, and (b) lipid phase separation [20]. It has also been reported that oleic acid is a powerful enhancer for transdermal delivery [14], as it increases the fluidity of the intercellular lipid barriers in the stratum corneum by forming separate domains which interfere with the continuity of the multilamellar stratum corneum and induce highly permeable pathways in the stratum corneum [15, 16]. From the above permeation study, it can be concluded that decreasing oil content, (from 23.26% to 4.00%), the transdermal flux increases from (30.10 to 211.812 µg/cm²/h) which was 7.03 fold greater than nanoemulsion having 23.26 % oil phase.

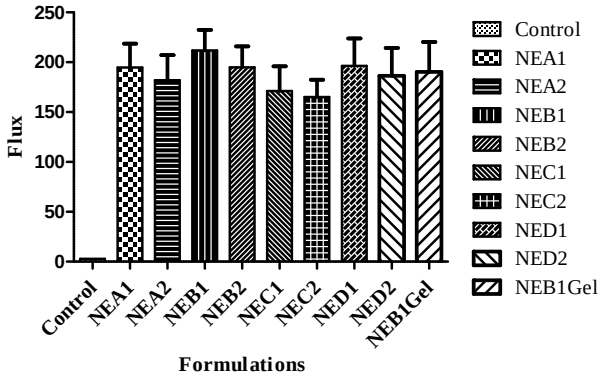

Figure 2: Transdermal Flux of different nanoemulsion formulations

The transdermal flux of carvedilol saturated oil phase was too less 2.8641, this may be due to large content of carvedilol may have substantially reduced partition coefficient between the skin and vehicle for the drug, which can counteract the benefit of the increased concentration gradients effect, and thereby actually decrease the transdermal flux.

From the above study, it can be also concluded that formulation containing a lower amount of S/Cos, provide higher flux than formulation containing higher amount of S/ CoS. This may be due to an increased thermodynamics activity of the drug in nanoemulsion formulation at lower concentration of surfactant and cosurfactant [14].

The flux of nanoemulsion gel of NEB1Gel was found 190.2591±25.58 µg/cm²/h which showed slightly less than NEB1, 211.8123±20.76 µg/cm²/h but significantly very high than drug saturated oil phase (shown in figure 2), the decrease in flux as compared to NEB1 might be due to change in the water content and viscosity of nanoemulsion gel formulation. Cumulative amount of carvedilol permeated from through rat skin was shown in figure 3, where Cumulative amount of carvedilol permeated of NEB1

is greater than the NEB1Gel. On the basis of *in-vitro* studies it was found that required area 2.97 cm² on human skin would provide the desired steady state conc. in the plasma.

The conductivity test show that the nanoemulsion was o/w type since a current flow was observed.

It was found that drug showed a solubility of 34.57 mg/ml in the nanoemulsion which is about73.95 fold higher than solubility of drug in water (2.8641 mg/ml) which leads to a greater concentration gradient towards skin leading to greater flux across the skin.

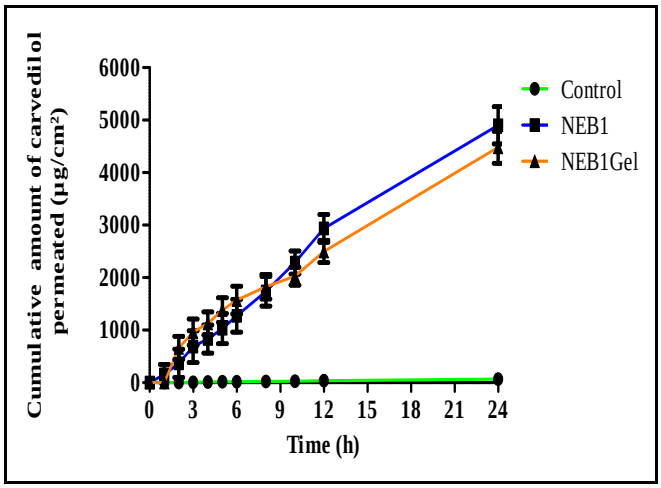

Figure 3: Cumulative amount of carvedilol permeated from nanoemulsion formulations through rat skin

The formulation with time reduces their stability hence drug loaded nanoemulsion were kept for 3 months and their drug retained capacity was calculated. Stability study was applied on optimized nanoemulsion formulation. Stability studies according to ICH guidelines at 75% RH predicted a degradation of 1.635% of carvedilol at the end of 90 days. The results show that formed nanoemulsion is stable.

The skin irritancy test was performed to confirm the safety of the nanoemulsion gel formulation. Utely and Van Abbe et al., (1975) mentioned that a value of skin irritancy score between 0 and 9 indicates that the applied formulation is nonirritating and safe for human skin. The mean value of skin irritancy score for formulation nanoemulsion gel (NEB1) was found to be 0.142. From this can be concluded that the prepared nanoemulsion formulation was safe to be used as transdermal drug delivery system.

Table 5. Pharmacokinetic parameters of nanoemulsion formulations

Parameters	Control	Oral	Nanoemulsion Gel (NEB1Gel)
C_{max} (µg/ml)	2.10	4.23*	3.48**
t_{max} (h)	3.01	2.00	12.00
AUC_{o-t} (µg/ml/h)	9.97	37.09*	63.89**
MRT (h)	5.90	7.27	10.88

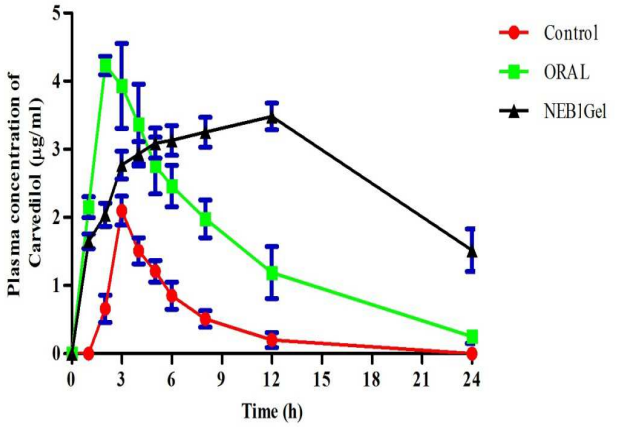

Figure 4: Carvedilol concentration in rat serum after oral and transdermal treatment.

One way ANOVA (Dunnet Multiple comparison test) AUC_{o-t} NEB1Gel significantly higher than control of Carvedilol ($P^* < 0.05$, $P^{**} < 0.003$).

Based on higher drug release, optimum droplet size and lower viscosity nanoemulsion formulation gel (NEB1 gel) was selected for the *in-vivo* study. The above figure inform the pharmacokinetic profiles of carvedilol after topical application of drug loaded nanoemulsion gel formulation as compared to control formulation and orally carvedilol tablet. The C_{max} of nanoemulsion (NEB1Gel) was found to be 3.48µg/ml and t_{max} was 12h. The $AUC_{o\rightarrow t}$ of topically applied nanoemulsion gel formulation was 1.72 fold higher compared to orally administered carvedilol tablet. After oral administration the plasma levels of carvedilol was reached at a peak of 4.23 µg/ml at 2.0

h while topical administration of nanoemulsion gel formulation and control formulation reached a peak of 3.48 and 2.10 µg/ml at 12.0 and 3.01 h respectively. Nanoemulsion gel formulation showed the low C_{max} and prolonged $t_{max,}$ which was due to the barrier properties of the skin and slow portioning of carvedilol into the skin from NEB1Gel. The highest MRT values of NEB1 gel versus oral route might be due to continuous replenishment of drug into the systemic circulation by controlled delivery of drug from the nanoemulsion.

Conclusion

Thus transdermal application of NEB1Gel (which is consisting of Oleic acid+IPM3:1, Tween-20 and Carbitol) has a sustained and enhanced absorption. The nanoemulsion formulation (NEB1) of carvedilol containing 6 mg of carvedilol, 4% of oil phase (oleic acid: IPM 3:1), 12% of surfactant (Tween-20), 24% cosurfactant (carbitol) and 60% of double distilled water. From *in-vitro* and *in-vivo* data it can be concluded that the developed nanoemulsions formulation have great potential for transdermal drug delivery.

Carvedilol is a third generation $\beta_1+\beta_2+\alpha_2$ adrenoreceptor blocker; produces vasodilatation due to α_2 blocked as well as direct action, and has antioxidant property. It is indicated for the treatment of hypertension and mild or moderate heart failure. However it undergoes extensive first pass metabolism after oral administration. Approximately 75% of drug gets metabolized; leading to an absolute bioavailability of 23%. Therefore the transdermal route would be beneficial to improve its bioavailability by circumventing first pass metabolism.

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