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RESEARCH ARTICLE

# DEVELOPMENT AND EVALUATION OF NANOEMULSION FORMULATIONS FOR IMPROVED ORAL DELIVERY OF CARVEDILOL

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## Abstract

**Objective:** The aim of the present investigation was to develop, optimize and evaluate nanoemulsion system of carvedilol to improve its solubility, and oral bioavailability. Carvedilol is a non-selective beta blocker used in the treatment of mild to moderate congestive heart failure and mild to moderate essential hypertension. It has both poor water solubility (0.583 mg/L) and oral bioavailability (23%) because of significant first-pass hepatic metabolism.
**Methods:** Based on solubility testing, clove oil was used as oil, Tween 20 was used as surfactants and PEG 400 was used as cosurfactants in construction of phase diagrams. Carvedilol nanoemulsions were prepared by aqueous phase titration method. Out of twelve formulations, eight thermodynamically stable formulations were selected for preparation of carvedilol loaded nanoemulsions and these nanoemulsions were subjected for characterization i.e. particle size, viscosity, polydispersity, zeta potential. A 12 hrs *in-vitro* release release study was performed on selected nanoemulsion formulations of carvedilol.
**Results:** The results of viscosity of carvedilol nanoemulsions were found to be in range (60.42 –134.63 m Pa.sec.). The results of pH measurement for formulations explain that the pH values of drug free nanoemulsions were slightly acidic. All formulations have PDI value less than (1.0).
**Conclusion:** Study concludes, nanoemulsion formulation of batch NEC4 ($S_{mix}$ ratio 1:3) was found to be optimum formulation.
**Keywords:** Bioavailability, Carvedilol, first-pass hepatic metabolism, nanoemulsion.

## INTRODUCTION

The term 'nanoemulsions' is used to designate emulsions with the internal phase droplets having size ranging from 50 to 1000 nm[1]. Nanoemulsions are dispersed particles used for pharmaceutical and biomedical aids and vehicles. Size of the droplets is governed by the surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point induced by either temperature or composition[2]. They are composed of an oil phase, aqueous phase, surfactant and co surfactant at appropriate ratios. The particles can exist as water in-oil and oil-in-water forms, where the core of the particle is either water or oil, respectively[3].
Nanoemulsions are based on low interfacial tension, achieved by adding a co-surfactant, which leads to spontaneous formation of a thermodynamically stable nanoemulsion. Nanoemulsions have high kinetic stability, low viscosity, and transparency/translucency, are very attractive for a range of industrial applications, including the pharmaceutical field where they have been explored as drug delivery systems. The nanoemulsions are also termed as mini emulsions, ultrafine emulsions and submicron emulsions[4].
Carvedilol is a non-selective beta blocker used in the treatment of mild to moderate congestive heart failure and mild to moderate essential hypertension[5]. It is a poor water- soluble and highly permeable drug. Its oral bioavailability is low (23%) because of significant first-pass hepatic metabolism by cytochrome P450[6,7]. However, some sources suggest that this low bioavailability is the result of poor aqueous solubility. It is practically insoluble in water (0.583 mg/L) and has pH-dependent solubility. Carvedilol also has a short plasma half-life of 7-10 hrs. Many parameters like low oral dose (6.25–25.0 mg), suitable log P (octanol/water) of 4.19, low oral bioavailability and the

condition of being a BCS class II drug make it suitable candidate to use it for the development of nanoemulsions[8]. The aim of this study was to assess the feasibility of preparing Carvedilol nanoemulsion by aqueous phase titration method, and the physicochemical properties of obtained Carvedilol loaded nanoemulsions, such as particle size, viscosity, polydispersity, zeta potential, *in vitro* drug release behavior.

## MATERIALS AND METHODS

Carvedilol was obtained from Olex pharmaceuticals, Nigeria as gift sample. Castor oil, clove oil, silicon oil were purchased from Omolad Oli Nig Ltd, Nigeria. PEG-600, PEG-400, PEG 200, 80, Tween 20, Span 60 were purchased from Agmont Industries Nig Limited, Nigeria.

**Screening of oils, surfactants and co-surfactants for nanoemulsion**

**Solubility studies**

The solubility of Carvedilol in various oils (Capryol 90, Isopropyl myristate, Oleic acid, Olive oil, Sunflower oil and Linseed oil), surfactants (Tween 20 and Tween 80) and cosurfactants (Transcutol P, Propylene glycol, PEG 400 and Glycerol) was determined by adding an excess amount of drug in oils, surfactants and co surfactants separately in stopper vials, and mixed[9]. The mixture vials were then kept at 25±1.0°C in an Orbital shaker for 72 hrs to reach equilibrium. The samples were removed after achieving equilibrium and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45-µm membrane filter. The filtrate was solubilized in suitable solvent, diluted with the pH 7.4 buffer and the concentration of Carvedilol was determined using UV-Visible spectrophotometer (Finlab Ltd, Nigeria) at 241 nm.

**Selection of Surfactant**

Surfactant selection was done on the basis of percentage of transparency (% transparency) and ease of emulsification. Briefly, 0.3 ml of each surfactant was added to the selected 0.3 ml of oil phase[10]. The mixture was gently heated at 50ºC for homogenization of the components. Each 0.05 ml mixture was then diluted with water in a stoppered conical flask. Ease of emulsification was judged by the number of flask inversion required to yield a homogenous emulsion. Emulsion is allowed to stand for 2 hours and their % transparency was evaluated by UV spectrophotometer using distilled water as a blank at 241 nm.

**Selection of Co-surfactant**

Screening of co-surfactant was conducted on the basis of % transparency and ease of emulsification. 0.1 ml of each cosurfactant mixed with 0.2 ml of selected surfactant and the 0.3 ml of selected oil phase was added and evaluated in a similar fashion as described in the above section of surfactant[11].

**Construction of phase diagrams**

Pseudo ternary phase diagrams were constructed for 1:1, 1:2 and 1:3 surfactants to cosurfactant ratios ($S_{mix}$). So that nanoemulsion regions could be identified. In construction of phase diagrams clove oil was used as oil, Tween 20 was used as surfactants and PEG 400 was used as cosurfactant[12]. Nanoemulsions were prepared by aqueous phase titration method. The composition of the nanoemulsions was chosen according to the pseudo ternary phase diagram. The drug was dissolved in the oil, surfactant and co-surfactant mixture was added in the chosen concentration, and water was added drop wise with continuous stirring until clear nanoemulsion was formed. Resulting carvedilol containing nanoemulsion was subjected to homogenization for 10 minutes using ultra turrex (Silverson, U.K) at 8000 rpm to get uniform and stable nanoemulsion[13].

**Characterization of nanoemulsions**

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nanoemulsions from emulsions that have kinetic stability and will eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests.

**a). Centrifugation test:** Nanoemulsions were centrifuged for 20 min at 3000 rpm and checked for phase separation, creaming or cracking[14].

**b). Freezing–thawing test**: The formulations were subjected to two different temperatures which are (21°C) and (-21°C) using refrigerator and the time for each temperature not less than 24 hours. This test used to indicate accelerated stability of formulations[15].

**c). Heating-cooling test**: This test was done by keeping the formulations at 40°C and at 0ºC by refrigerator for 48 hours. This test used to indicate the racking effect on the formulations stability[15].

Optimized formulations were taken for viscosity, refractive index, transmittance and *in vitro* release studies.

**Preparation of Carvedilol loaded nanoemulsion:**

Carvedilol nanoemulsions were produced by dissolving the quantity of drug in specialized amount of oil. Then the determined quantity of $S_{mix}$ added for oil loaded drug, after that the whole mixture was blended together by vortex mixer (Mon Scientific, Nigeria), at the speed of 100 rpm. Then the aqueous phase (deionized water) titrated drop by drop to obtain transparent, clear (o/w) nanoemulsion.

**Particle size and zeta potential (ZP) measurement**

An amount of 0.1 ml of each tested formulation was dispersed in 50 ml of water in volumetric flask and then mixed by inverting the flask. Globule size and zeta potential of the nanoemulsion was determined by particle size analyzer (QL-1076, Nigeria) that analyzes the fluctuations in light scattering due to Brownian motion of the particles. Light scattering was monitored at 25°C at a 90°C angle[16].

**Poly dispersity index (PDI) assay**

This assay is used to measures the uniformity of globules size in nanoemulsion. It can be obtained by ABT-9000 nanolaser particle size analyzer. The higher

the poly dispersity value refers to the lower uniformity of globules size of nanoemulsion[17].

**Determination of pH**

The pH value plays important role in determination of the stability of the nanoemulsion. Change in pH means occurrence of chemical reactions that can impair the quality of the final product. The digital pH meter was used to determine the pH of the formulations.

**Refractive Index**

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

**Viscosity**

Viscosity of the samples was measured as such without dilution using Brookfield viscometer at 25°C. A sample volume of 10 ml was used. The nanoemulsion formulations were subjected to different rpm (5, 10, 20, 30, 50, 60 and 100) and the rheological behavior of the disperse system was examined by constructing rheograms of shear stress vs. shear rate[18].

**Drug content**

Drug content of RF in nanoemulsion was measured by using UV visible spectrophotometer at 241 nm. About 0.1 mL of the formulation was suitably diluted with 5 mL in pH 7.4 phosphate buffer and analyzed for drug content[19].

***In vitro* drug release studies**

The *in vitro* drug release of Carvedilol from the nanoemulsion formulation was determined by dialysis bag method[20]. The dissolution study was performed for 12 hrs. In this method 0.1N HCl and pH 7.4 phosphate buffer maintained at 37°C and stirred with a magnetic stirrer (STUART, Finlab, Nigeria) was selected as *in vitro* release medium. About 1 mL of formulation was placed in the dialysis bag which was immersed in 50 mL of 0.1 N HCl. Samples (2 mL) were withdrawn at predetermined time intervals and replenished with equal volume of fresh medium. The samples were analyzed by the UV-Visible spectrophotometer at 241 nm to determine the Carvedilol content[21].

**Kinetics models and drug release mechanism**

Determination of release kinetic of drug was done by using various kinetics models. The results of dissolution must be fitted for these kinetics models which are (zero order kinetic, first order kinetic, Higuchi model)[22].

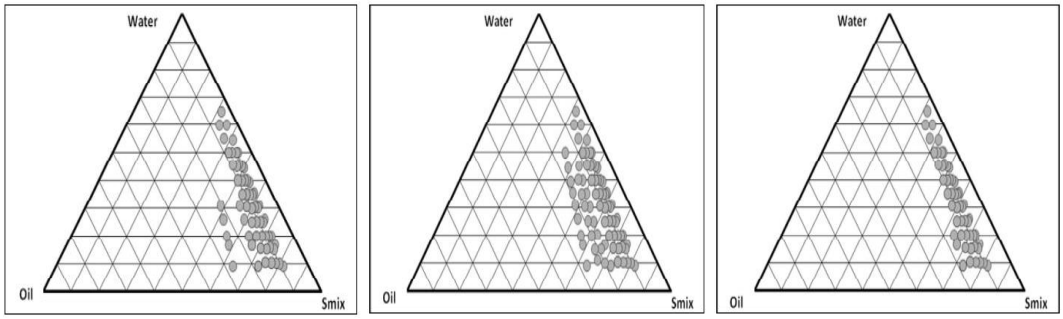
**Statistical analysis**

All the data generated were expressed as mean±standard deviation. Three group comparisons, one-way analysis of variance with duplication was applied. Statistical significance was determined using Student's *t*-test, with $p<0.05$ considered to be statistically significant.

**Table 1: Solubility of carvedilol in different oils, surfactants and cosurfactants.**

| Oils | Solubility (mg/ml) | Surfactants | Solubility (mg/ml) |
|---|---|---|---|
| Silicon oil | 0.48±0.18 | Span-80 | 46.58±0.38 |
| Eucalyptus oil | 4.62±0.39 | Tween-80 | 28.94±0.49 |
| Clove oil | 159.63±1.4 | Tween-20 | 84.94±0.97 |
| Castor oil | 16.38±0.91 | **Cosurfactants** | **Solubility (mg/ml)** |
| Olive oil | 9.35±0.38 | PEG-600 | 141±1.7 |
| Capmul MCMC8 | 29.53±0.84 | PEG-400 | 230.57±0.64 |
| Isopropyl myristate (IPM) | 11.48±0.66 | PEG-200 | 189.08±0.38 |
| Triacetin | 11.89±1.59 | Propylene glycol | 9.56±0.89 |

Mean±SD, n=3




(a). $S_{mix}$ 1:1 (b). $S_{mix}$ 1:2 (c). $S_{mix}$ 1:3

**Figure 1: Pseudo Ternary phase diagram of clove oil, Tween 20, PEG 400 and water.**

## RESULTS AND DISCUSSION

Drug solubility in oils plays an important role, as the ability of the nanoemulsion to maintain the drug in solubilized form depends on the solubility of the drug in the oil phase. In case of inadequate solubilization, there may be chances of precipitation, particularly in case of oral or parenteral nanoemulsion. After screening the oils for carvedilol solubility, it was found that carvedilol exhibited maximum solubility in clove oil (Table 1). Hence clove oil was chosen as the oil phase. Similarly based on solubility parameter Tween 20 was used as surfactants and PEG 400 was used as cosurfactant. The components of pseudo-ternary phase plot (Figure 1) are oil, water and $S_{mix}$ (surfactant /co surfactant) which considered as variable component due to that it presents in different ratio such as 1:1, 1:2, 1:3 as shown in Figure 1. The shaded area represents

the area of nanoemulsion and the larger shaded area indicates a good nanoemulsifying activity. Based on the results of thermodynamic stability tests as shown in Table 3, eight formulations were selected for preparation of carvedilol loaded nanoemulsions and these nanoemulsions were subjected for characterization. Different results are shown in Table 4, i.e. droplet size, PDI, pH and % transmittance etc. For carvedilol loaded nanoemulsions explains that the droplets size was decrease with increase in the $S_{mix}$ ratio. The results of PDI as shown in Table 4 indicate the uniformity of droplets distribution within the formulations. All formulations have PDI value less than (1.0). The lower value of PDI was (0.088) for NEC2, this explains that NEC2 has higher uniformity of droplets distribution within formulation. The results of viscosity of carvedilol nanoemulsions were found to be in range (60.42 –134.63 m Pa.sec.). Viscosity has an important aspect to ensure the smoothen formulations, packing[23]. The refractive index of the formulated nanoemulsion was similar to the refractive index of the water (1.333).

**Table 2: Different $S_{mix}$ ratio (clove oil, Tween 20, PEG 400).**

| Oil : S/ COS | Formulation code | Oil (mg) | Surfactanct (mg) | Cosurfactant (mg) | Water (% w/w) |
|---|---|---|---|---|---|
| | | | **$S_{mix}$ ratio 1:1** | | |
| 0.5:9.5 | NEA1 | 0.050 | 0.420 | 0.525 | 54.5 |
| 2:8 | NEA2 | 0.198 | 0.370 | 0.396 | 56.7 |
| 4:6 | NEA3 | 0.396 | 0.295 | 0.295 | 60.00 |
| 6:4 | NEA4 | 0.594 | 0.385 | 0.196 | 59.25 |
| | | | **$S_{mix}$ ratio 1:2** | | |
| 0.5:9.5 | NEB1 | 0.050 | 0.314 | 0.626 | 52.84 |
| 2:8 | NEB2 | 0.198 | 0.264 | 0.528 | 57.52 |
| 4:6 | NEB3 | 0.396 | 0.198 | 0.396 | 51.35 |
| 6:4 | NEB4 | 0.594 | 0.132 | 0.264 | 52.66 |
| | | | **$S_{mix}$ ratio 1:3** | | |
| 0.5:9.5 | NEC1 | 0.050 | 0.626 | 0.314 | 54.32 |
| 2:8 | NEC2 | 0.198 | 0.528 | 0.264 | 57.22 |
| 4:6 | NEC3 | 0.396 | 0.396 | 0.198 | 58.12 |
| 6:4 | NEC4 | 0.594 | 0.145 | 0.132 | 60 |

**Table 3: Thermodynamic stability tests for nanoemulsion formulations of pseudo ternary phase diagram.**

| Code | Thermodynamic stability test | | | Results |
|---|---|---|---|---|
| | Centrifuge | Freeze thawing | Heating-cooling | |
| NEA1 | ✓ | ✓ | ✓ | Pass |
| NEA2 | ✗ | ✓ | ✗ | Fail |
| NEA3 | ✓ | ✓ | ✓ | Pass |
| NEA4 | ✓ | ✓ | ✓ | Pass |
| NEB1 | ✓ | ✓ | ✗ | Fail |
| NEB2 | ✓ | ✓ | ✓ | Pass |
| NEB3 | ✓ | ✓ | ✓ | Pass |
| NEB4 | ✗ | ✓ | ✗ | Fail |
| NEC1 | ✓ | ✓ | ✓ | Pass |
| NEC2 | ✓ | ✓ | ✓ | Pass |
| NEC3 | ✓ | ✗ | ✓ | Fail |
| NEC4 | ✓ | ✓ | ✓ | Pass |

The results of % transmittance explain that the formulated nanoemulsions were clear and transparent, and the transparency of nanoemulsions indicates that the droplets size was in nano-scale. The higher value of % transmittance in carvedilol loaded nanoemulsions was (98.68 %) for NEC4. The results of pH measurement for formulations explain that the pH values of drug free nanoemulsions were slightly acidic. The higher pH value in the formulations of carvedilol nanoemulsions was (6.23) for NEA3 and this pH value is suitable for oral administration. Zeta potential of different formulations was calculated to explain the electro kinetic potential in colloidal dispersions. The results of zeta potential of carvedilol nanoemulsions were in range -18.34 mV to -26.47 mV).

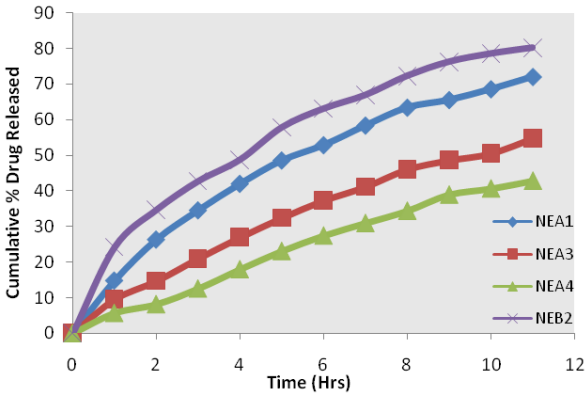



**Figure 2: *In vitro* dissolution profiles of Carvedilol nanoemulsion formulations of batch NEA1-NEB2.**

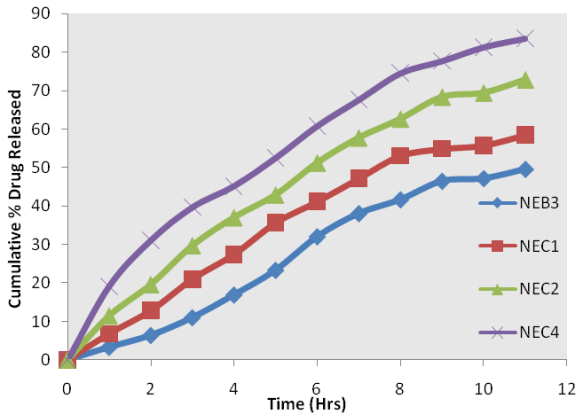



**Figure 3: *In vitro* dissolution profiles of Carvedilol nanoemulsion formulations of batch NEB3-NEC4.**

According to rule of thumb, the values of zeta potential which are: range -5 mV to +5 mV indicate fast aggregation, about -20 mV or +20 mV provide short term stability, above +30 mV or below -30 mV indicate a good stability and above +60 mV or below -60 mV offers excellent stability[24]. The results of drug content in nine formulations of carvedilol loaded nanoemulsions were in range (92.1-98.9%). The higher percent of drug content (99.04 %) was found in NEA4 that has $S_{mix}$ (1:1), and the lowest percent of drug content (96.48 %) was found in NEC1 that has $S_{mix}$ (1:3). A 12 hrs *in-vitro* release study was performed on selected nanoemulsion formulations of carvedilol (Figure 2 and Figure 3). The highest *in-vitro* release is shown by formulation of batch NEC4 (84.57 %), while the lowest release is shown by NEA4 (42.786 %). Analysis of variance (ANOVA), study was performed by means of ezANOVA software. It reveals that there was a significant difference ($p<0.05$) between the release of carvedilol in all formulations. The release of drug from carvedilol nanoemulsions in dissolution media explains the influence of surfactant concentration on the release of carvedilol for each ratio of $S_{mix}$. In each $S_{mix}$ ratio, as the concentration of Tween 20 increase, the release of carvedilol decrease. This may be due to increasing the concentration of Tween 20 results in increasing the diffusion of carvedilol molecules from dialysis bag for the dissolution medium[15].

**Table 4: Droplet size, polydispersity, viscosity, and RI of nanoemulsion.**

| Code | Droplet size (nm) | Polydispersity index (PDI) | Viscosity (mPa s) | RI | % Transmittance | ZP (Mv) | pH | % Drug content |
|---|---|---|---|---|---|---|---|---|
| NEA1 | 80.81±0.85 | 0.163 | 60.42±0.88 | 1.312±0.006 | 95.42±0.08 | -18.34±1.46 | 5.43±0.08 | 98.46±0.04 |
| NEA3 | 78.47±2.4 | 0.167 | 120.45±2.7 | 1.302±0.009 | 98.24±0.13 | -21.54±3.41 | 6.23±0.07 | 97.64±0.12 |
| NEA4 | 77.39±0.77 | 0.093 | 128.58±1.5 | 1.334±0.008 | 97.57±0.09 | -22.66±2.58 | 5.51±0.04 | 99.04±0.08 |
| NEB2 | 74.45±3.6 | 0.173 | 134.63±3.8 | 1.327±0.007 | 96.89±0.04 | -25.37±1.49 | 6.13±0.12 | 98.27±0.05 |
| NEB3 | 73.48±4.8 | 0.094 | 129.34±4.7 | 1.315±0.004 | 97.33±0.21 | -26.47±2.37 | 5.72±0.21 | 99.23±0.01 |
| NEC1 | 70.85±0.68 | 0.166 | 129.42±3.8 | 1.408±0.009 | 94.32±0.57 | -24.11±2.64 | 5.33±0.09 | 96.48±0.04 |
| NEC2 | 69.07±1.6 | 0.088 | 98.53±2.6 | 1.351±0.003 | 96.22±0.82 | -25.73±1.39 | 6.21±0.07 | 98.72±0.06 |
| NEC4 | 68.42±2.8 | 0.164 | 60.53±1.8 | 1.293±0.006 | 98.68±0.18 | -22.39±0.82 | 5.66±0.31 | 97.65±0.07 |

The data of dissolution were fitted for various kinetic models (Table 5). It was found that the higher regression coefficient ($R^2$) values in the zero order kinetic. So, the kinetic of drug release in all Nanoemulsions was zero- order kinetic and the values of diffusion exponent (n) for all nanoemulsions of carvedilol was significantly lower than 0.41 ($p<0.05$), this explains that the mechanism of release of nimodipine from nanoemulsion formulations was Fikian release (diffusion).

**Table 5: Values of regression coefficient ($R^2$) and values of diffusion exponent (n).**

| Code | Zero order kinetic | First order kinetic | Higuchi model | Diffusion exponent |
|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | N |
| NEA1 | 0.972 | 0.915 | 0.923 | 0.26 |
| NEA3 | 0.986 | 0.938 | 0.953 | 0.28 |
| NEA4 | 0.967 | 0.945 | 0.964 | 0.41 |
| NEB2 | 0.987 | 0.963 | 0.954 | 0.29 |
| NEB3 | 0.978 | 0.972 | 0.895 | 0.36 |
| NEC1 | 0.983 | 0.942 | 0.948 | 0.28 |
| NEC2 | 0.982 | 0.961 | 0.925 | 0.14 |
| NEC4 | 0.985 | 0.972 | 0.935 | 0.34 |

($p<0.05$)

## CONCLUSIONS

Nanoemulsions are considered as an advance technique for improving the bioavailability of poorly water - soluble drugs by enhancing the solubility and minimizing the first pass metabolism. A correct combination of oil, surfactant, cosurfactant, and water is a major consideration factor in nanoemulsion preparation. Clove oil, Tween 20, PEG 400 were selected for preparation of nanoemulsion by aqueous phase titration method. On the basis of different *in-vitro* release study, carvedilol nanoemulsion formulation of batch NEC4 ($S_{mix}$ ratio 1:3) was found to be optimum formulation. The optimized formulation showed low particle size, low viscosity and high

percentage transmittance. The present study was clearly indicated that the usefulness of nanoemulsion in the improvement of the solubility, dissolution rate and there by oral bioavailability of carvedilol.

## AUTHOR'S CONTRIBUTION

**Edenta C:** designed the study. **Ezeaku IN:** acquired the data. **Zainab A:** analyzed the data and interpreted the results. **John DF:** drafted the article. All authors revised the article and approved the final version.

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## DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

## CONFLICT OF INTERESTS

None to declare.

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