Biomedical Sciences and Clinical Medicine 2025;64(4):277-295.
doi: 10.12982/BSCM.2025.33
https://he01.tci-thaijo.org/index.php/CMMJ-MedCMJ/index

Original Article

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Quality By Design Enabled Carvedilol Self-Nanoemulsifying Drug Delivery System: *In Vitro* and *In Vivo* Characterization for Improved Management of Hypertension

Rajib Lochan Maharana[1], Surya Kanta Swain[2], Santosh Kumar Mahapatra[3], Debashish Ghose[4] and Bikash Ranjan Jena[5]

[1]Research Scholar, Faculty of Pharmacy, Affiliated to Biju Patnaik University of Technology, Chhend Colony, [2]Amity Institute of Pharmacy, Newtown, AA-II, Kolkata, West Bengal, [3]Department of Pharmaceutics, College of Pharmaceutical Sciences, Puri-Konark Marine Drive Road, Baliguali, [4]Department of Pharmaceutics, Roland Institute of Pharmaceutical Sciences, Berhampur, [5]School of Pharmacy and Life, Centurion University of Technology and Management, Bhubaneswar, Odisha, India

Correspondence:
Surya Kanta Swain, PhD,
Amity Institute of Pharmacy,
Amity University, Kolkata,
Newtown, AA-II, Kolkata-700135,
West Bengal, India.
E-mail: swain_suryakant@yahoo.co.in

Received: February 3, 2025;
Revised: June 23, 2025;
Accepted: July 14, 2025

ABSTRACT

OBJECTIVE Due to poor absorption and substantial hepatic first-pass metabolism, carvedilol, a commonly used cardiovascular drug for hypertension and congestive heart failure, has a low and variable bioavailability. In order to enhance carvedilol solubility and prevent the first-pass impact, the current research work intends to produce SNEDDS of the drug using systematic DoE. This will ultimately increase its bioavailability.

METHODS By choosing the critical process parameters as factors that influenced the intended responses, quality by design was made possible. Oil and S/CoS were examined for pre-isotropic compatibility and formulation enhancement using Design of Experiment software. The cumulative percentage of drug release (QT30) in minutes, emulsification time (ET) in minutes, and emulsion globule size (nm) of the nano formulations were measured using a heating-cooling cycle and phase separation. To enhance carvedilol biopharmaceutical efficacy and oral bioavailability for the therapeutic management of cardiovascular disease. The carvedilol loaded self-nano emulsifying drug delivery system has been prepared by admixture method with selected oil, surfactant, and co-surfactant based on higher mean saturation solubility of drug. QbD approach presents an effective method to develop SNEDDS formulations of carvedilol with enhanced Using Design of experiments software, oil (Lauroglycol FCC), surfactant (Tween 20), and co-surfactant (Propylene glycol) were optimized for pre-isotropic compatibility and formulation development.

RESULTS The prepared SNEDDS exhibited non-Fickian mechanism of drug release, according to *in-vitro* drug release kinetic data. The optimised formulation had uniform shape and nanosize with no physical incompati-bilities between the selected excipients and the pure drug based on SEM, DSC and FT-IR. After six months of storage, remains stable, according to accelerated stability tests.

CONCLUSIONS In order to improve the therapeutic treatment of cardiovascular disease, the QbD approach presents an effective method to develop SNEDDS formulations of carvedilol with enhanced oral bioavailability and biopharmaceutical performance.

KEYWORDS pseudo-ternary phase diagram, quality by design, SNEDDS, hypertension, *in-vitro* drug release, bioavailability, stability

INTRODUCTION

Globally, cardiovascular diseases (CVDs) account for around 30.00% of all deaths, making them the most common category of severe diseases (1, 2). Oral administration of cardiovascular medications has unquestionably been the most sought-after goal of both patients and manufacturers, despite significant advancements in innovative drug delivery systems (DDS) via alternate routes (3). Its widespread acceptance, superior safety compared to the parenteral route, lower therapeutic costs, and increased patient compliance are the main reasons. As a result, oral DDS now make up over 80.00% of the commercially available cardiovascular DDS (4). A common medication used to treat a variety of CVDs is carvedilol, which is recommended not only for hypertension but also for myocardial infarction and congestive heart failure. Given its log p of 4.115 and its limited water solubility, it can be safely classified as a BCS class II medication (5). It also passes through a lot of first-pass metabolism in the liver, which causes humans and animals, such as dogs and rats, to have a much lower absolute oral bioavailability (20.00%) (6).

In this context, self-nanoemulsifying drug delivery systems (SNEDDS) have been receiving international recognition for their capacity to circumvent the hepatic first-pass effect and to enhance the bioavailability potential of lipophilic medicines by increasing their dissolution and penetration (7). Additionally, it has been shown that the SNEDDS play a significant part in overcoming the effects of intestinal metabolism via cytochrome P450 isoforms and P-gp efflux. The emulsions from the SNEDDS, which are isotropic combinations of lipids, emulsifiers, and co-emulsifiers, usually have a globule size that ranges from a few nanometres to several microns. In the GI tract, the SNEDDS formulation typically instantly produces a transparent dispersion that remains steady when diluted (8, 9). Depending on the size of the globules in the SNEDDS formulation, these dispersions are called either nanoemulsions or microemulsions. Self-nanoemulsifying SNEDDS are formulations that produce nano-sized globules. Using the conventional drug delivery methods of altering one variable at a time (OVAT) is not a feasible strategy for designing an ideal SNEDDS formulation as it calls for logical combinations of disparately acting lipids, surfactants, co-surfactants, charge inducers, etc. (10). On the other hand, systematic optimization of such isotropic delivery systems via design of experiments (DoE) provides several benefits, such as high accuracy and forecasts as well as cost, time, and effort savings (11, 12).

METHODS

Materials

A gift sample of carvedilol was provided by M/s Matrix Laboratories Ltd. (Hyderabad, India). Gift samples of Labrafil M (Lauroyl polyoxyl-6 glycerides), Lauroglycol FCC (Propylene Glycol Monolaurate), and Propylene Glycol Dicaprylocaprate (Labrafac PG) were obtained from M/s Gattefosse (Saint-Priest, France). Messrs. BASF GmbH of Minden, Germany, provided the Cremophor RH40 (PEG-40 Hydrogenated Castor Oil) free of charge. The following products were provided as complimentary samples from M/s Abitec Corp. of Janesville, Wisconsin, USA: Capmul MCM (Glyceryl caprylate/caprate) and Tween 20 (Polyoxyethylene sorbitan monolaurate-20). All additional materials, chemicals and solvents used in this research were of analytical grade.

Solubility study and determination of λ_{max} of carvedilol in 0.1N HCl

Labrafac PG, Lauroglycol FCC, Labrafil M, Cremophor RH40, and Capmul MCM were among the lipids tested for carvedilol equilibrium solubility. Each of the chosen vehicles had an excessive amount of carvedilol added to it, and the combination was constantly mixed for 72 h at 37 ± 1°C. The mixture was then centrifuged after equilibrium had been attained, and the resulting supernatant was filtered employing a membrane filter with a pore size of 0.45µ (M/s mdi Membrane Technologies LLC, Pennsylvania-17011, California, USA). The filtrate absorbance was measured at λ_{max} 287 nm using a double beam UV-Visible spectrophotometer (UV 3000+, M/s Labindia, Mumbai, India) (13-17). A quantity of 100 mg of the pure drug was diluted in 100 mL of a pH 0.1N HCl buffer at a concentration of 1,000 µg/mL. 1 mL of this solution was placed in a 10 mL volumetric flask and filled to volume with pH 0.1 N HCl. The solution was diluted with pH 0.1 N HCl buffer to create a series of dilutions comprising 10,

20, 30, 40, 50, 60, 70, 80, and 90 µg/mL of pure drug. The absorbance of the aforementioned dilutions was measured at λ_{max} 287 nm using a double beam UV-Visible spectrophotometer, using 0.1N HCl buffer as a blank. After that, a straight line was produced by plotting absorbance against concentration on the Y-axis (18-20).

Selection of surfactants and co-surfactants and construction of a pseudo-ternary phase diagram

For accurate boundary delineation between the nanoemulsion and emulsion phases, several oil-to-surfactant ratios were used within the range. Visual observations were taken and documented after each addition of 5 mL of water. (21) In order to visually examine their self-emulsifying characteristics, a series of SNEDDS were produced. The SNEDDS formulations were optimized by constructing pseudo-ternary phase diagrams in the absence of carvedilol to identify the self-emulsifying regions and to determine the optimal concentration of oil, surfactant, and cosurfactant. The phase diagrams of the systems with Tween 20 as the surfactant, Lauroglycol FCC as the oil, and Propylene glycol as the co-surfactant. With a S/CoS concentration of more than 65.00% of the SNEDDS formulation, emulsification efficiency was found to be excellent. The spontaneity of the self-emulsification process was shown to increase when the concentration of the surfactant Tween 20 inside the self-emulsifying zone was increased. It was noted that emulsification was inefficient when the surfactant ratio was less than 50.00%. The nanoemulsion formulation was therefore based on a 2:1 ratio of Tween 20: Propylene glycol to Lauroglycol FCC. Reportedly, the self-emulsifying performance may be affected by the drug included in the SNEDDS particles. In comparison with the self-emulsifying performance of the respective formulations with and without carvedilol, our results showed no statistically significant changes. Nanoemulsion generation may be pinpointed using a ternary diagram which was created to show the precise ratio of surfactant to co-surfactant (22-24).

Quality target product profile (QTPP) and Critical quality attributes (CQAs) Identification

Quality by design (QbD) was utilized to prepare the SNEDDS formulations. Table 1 shows QTPPs and CQAs for developing carvedilol-loaded SNEDDS. To fulfil the QTPP, nanoemulsion formulations and physical properties were specified as essential CQAs. Cumulative drug release QT30 (%), emulsification time (minutes), and globule size (nm) were key product performance indicators (25, 26).

Preparation of self-nanoemulsifying drug delivery systems

The SNEDDS formulations were made by the usual admixture process. The initially required concentration of the drug was dissolved in the selected lipid at room temperature. The predetermined ratio of surfactant and co-surfactant were added to the prepared lipidic drug solution while stirring with a magnetic stirrer at constant speed of rotation at ambient temperature. Based on the pseudo-ternary phase diagram's maximum nanoemulsion area, Lauroglycol FCC as oil (X1), Smix ratio (X2), and Tween 20 (X3) as surfactants were chosen for formulation optimiza-

Table 1. QTPPs and CQAs for developing carvedilol loaded SNEDDS

QTPPs	Target	CQAs	Predetermined target	Justifications
Dosage type	Immediate dosage forms	Cumulative % drug release at 30 minutes (QT30)	≥ 80%-95%	Immediate release of drug is the objective of the study and is important for better drug absorption.
Dosage form	SNEDDS	Globule size (nm)	100-200 nm	Highly critical factor as its role in permeation and retention of bio actives in SNEDDS delivery.
Dosage Stability	Stability	Emulsification time	≤ 10 mins	Highly critical factor as its role in ensuring stability of the formulation.

QTPPs, quality target product profiles; CQAs, critical quality attributes; SNEDDS, self-nanoemulsifying drug delivery systems

tion. In the following investigations, appropriate surfactant and co-surfactant limits were determined. A Box–Behnken design (BBD) with α = 1 was used to study oil and surfactant quantities at each of three coded levels. The different formulation compositions of carvedilol-loaded SNEDDS in seventeen experimental runs as per BBD along with the obtained CQAs responses, coded and actual levels (-1, 0, and 1).

The response variables for present optimization investigations were cumulative percentage of drug release (QT30) in minutes (Y1), self-emulsification time in minutes (Y2), and globule size in nm (Y3) (26-28). Risk assessment studies were used to examine several quality aspects associated with SNEDDS. This approach used the Ishikawa fishbone diagram for the preparation of SNEDDS. The studies primarily examined the influence of diverse process parameters (PPs) on critical material attributes (CMAs) and how those CMAs subsequently impacted the anticipated CQAs of the SNEDDS. Key risk variables that significantly impacted the chosen CQAs were determined by failure mode and effects analysis (FMEA). To ascertain the risk priority number (RPN), an extensive literature study, review of other existing information, and brainstorming sessions were used to evaluate the material and process parameter characteristics based on severity (S), occurrence (O), and detectability (D). The rank order scores ranged from 1 to 10, as seen in Table 2 (27-30).

Determination of self-emulsification time and dispersibility

One gram of each formulation was added to a 500 mL 0.5% w/v SLS solution and stirred continuously at 50 rpm using a USP 31 Apparatus 2 (Lab India, DS 8000, Mumbai, India) at 37±0.5°C temperature. Emulsification time was the time needed to thoroughly and evenly distribute the system (31).

Formulation optimization and preparation of solid-SNEDDS by experimental design

Systematic DoE optimization experiments examined the cumulative percentages of drug release (QT30) in minutes (Y1), self-emulsification time in minutes (Y2), and globule size in nm (Y3). Design Expert ver. 13. (Stat-Ease, Minneapolis, MN, USA) was used to create a comprehensive second-order polynomial equation with interaction terms to link the researched answers with the analyzed factors. The data showed a polynomial regression for the analyzed answers and predicted the best formulation (32). Because of its superior oil adsorption abilities, the 500 µL optimised liquid SNEDDS formulation (F8) was converted into free-flowing solid SNEDDS using the optimum amount (250 mg) of porous carrier Sylysia 350. For better oil adsorption and to prepare a solid homogenous mass, the liquid SNEDDS formulation was poured onto the selected porous carrier (250 mg) in a small stainless-steel bowl and thoroughly stirred for 30 minutes. To create a uniformly free-flowing solid self-nanoemulsifying drug delivery system for additional *in vitro* and *in vivo* study, the formulation was run through a sieve (BSS 22) (32).

Characterization

Viscosity and heating-cooling cycle

A Brookfield viscometer (Brookfield Engineering Labs, Middleboro, Massachusetts, USA) was used

Table 2. Factor analysis of materials and process variables using FMEA tool during the development of carvedilol loaded SNEDDS

Process parameters	Risk priority number	Severity (S)	Occurrence (O)	Detectability (D)
Concentration of oil (Lauroglycol) (mg/mL)	392±0.05	8	7	7
Concentration of co-surfactant (tween 20)	336±0.01	8	6	7
Smix ratio of oil: water: co-surfactant	280±0.08	7	8	5
Concentration of propylene glycol (%) (v/v)	135±0.04	5	3	9
Stirring speed (rpm)	140±0.09	4	5	7
Stirring time (minute)	150±0.11	6	5	5
Stirring type	120±0.07	5	4	6
Sonication speed per time	168±0.12	6	4	7

n: No. of observations (n = 3)

FMEA, Failure mode and effects analysis; SNEDDS, self-nanoemulsifying drug delivery systems

to evaluate the viscosity of liquid SNEDDS preconcentrates and 100-fold diluted samples at 100 rpm with a CC3-14 spindle at 25°C temperature (33). Three heating and cooling cycles were conducted to evaluate the stability of the chosen formulation. The drug-loaded SNEDDS were exposed to 4°C for 24 h and 45°C for 24 h for this purpose. The cycles were repeated three times. The formulation was checked for phase separation and drug precipitation after each cycle (34).

Centrifugation and freeze thaw cycle

In order to investigate the impact of centrifugation stress on the liquid SNEDDS that were developed, 100 mg of drug-loaded SNEDDS were reconstituted in 10 mL of distilled water to produce nano-emulsions. These nano-emulsions were then centrifuged at 12,000 rpm for 15 minutes and analyzed for any drug precipitate or phase separation (35). For each temperature, three freeze-thaw cycles were conducted, with an incubation period of 24 h at between -20°C and 25°C. The stress of each cycle was assessed for any drug precipitation and phase separation (36).

Zeta potential analysis and dispersibility testing

The zeta potential of SNEDDS formulations were measured using a zetasizer. The procedure involves reconstituting 10 µL of SNEDDS in 10 mL distilled water to create nano-emulsions and analysing the zeta potential. Each characterisation was repeated three times (37). Monitoring dispersibility upon dilution is another way to measure formulation self-emulsification efficiency. Dispersibility was tested using USP apparatus type II (Copley, NG 42JY, Nottingham, UK). To dissolve each formulation, 1 mL was introduced into a 500 mL distilled water vessel at 37 ± 0.5°C with a paddle speed of 50 rpm. The formulations were then visually tested for emulsification and clarity, and *in vitro* performance was graded. Grade A: clear or slightly blue nanoemulsion formed in 1 min. Grade B: fast-forming nanoemulsion, somewhat less clear and bluish-white in 2 min. Grade C: fine milky emulsion (2 min). Grade D: dull, grayish, whitish, somewhat greasy emulsion with sluggish emulsification (> 2 min). Grade E: compositions with low emulsification and big oil droplets (38).

Characterization of solid-SNEDDS

FT-IR and Differential scanning calorimetry

The FT-IR spectra for the optimized S-SNEDDS formulation (F8) and physical drug and excipient combinations were obtained using potassium bromide (Shimadzu Analytical (India) Pvt. Ltd., Model No. Shimadzu IR affinity-1). The transmittance was calculated between 4,000 and 400 cm^{-1} to assess the interaction between the drug and the excipient. The peak matching was conducted to ascertain whether there was an interaction between the pure drug and the excipient (16). The drug conformance with the oil: surfactant was evaluated using a SHIMADZU DSC-60 (differential scanning calorimetry). Each 10 mg specimen was reheated in aluminum containers using dried nitrogen as the effluent gases. The physical combinations of the pure drug with the optimized F8 formulation were determined using DSC thermogram analysis.

Encapsulation efficiency

The drug entrapment efficacy was assessed following their separation from the solution via centrifugation. The estimation process involved the acquisition of 10mL of solid SNEDDS, which were then centrifuged at 50,000 revolutions per minute at a temperature of -4°C for one hour using a refrigeration centrifuge (Model: Eltec Lab RC 4815). During the centrifugation dialysis procedure, a portion of the unbound drug was eliminated. The liquid that remained after sedimentation was collected and analyzed using UV spectrophotometry at a wavelength of 287 nm (UV 1800, Shimadzu, Japan) to confirm the presence of unbound drug, as predicted in equation-1.

Powder X-ray diffraction and scanning electron microscopy

The research made use of a powdered x-ray diffractometer manufactured by Rigaku in Japan, specifically, the Smart Lab 9 kW model. The samples underwent p-XRD scanning after being exposed to nickel-filtered CuKa radiation (40 kV, 30 mA). This information was obtained by comparing the peak intensity over time (h) with both the pure drug and optimized solid SNEDDS formulation (F8). The optimized formulation batch was morphologically examined using a Scanning

$$\% \text{ Entrapment efficiency} = \frac{(\text{Total drug quantity} - \text{Quantity of free drug})}{(\text{quantity of total drug})} \times 100 \text{.....equation-1}$$

Electron Microscope (SEM) from Tokyo, Japan, specifically the JEOL JEM1230 type, which has an 80 kV accelerating voltage. The adhering substrate of carbon was covered with a copper plate and left exposed to a single droplet of dispersion for one minute. A filter paper tip was used to fully minimize the residual dispersion (39).

In vitro drug release studies and in vivo pharmacokinetic study

In vitro drug release studies used membrane diffusion or the dialysis method. This approach separates nanoformulation from the release media using dialysis membranes that allow free drugs but not nanoformulation. The dialysis sac technique included placing SNEDDS systems in the sac and placing them in medium reservoir containers (0.1N HCl) with little agitation using a shaker water bath or magnetic stirrer (Tarson, Multispin, India). For *in-vitro* release characterization, 0.1N HCl was utilized. The solid SNEDDS (25 mg carvedilol) were suspended in the diffusion medium at 37 ± 0.5°C and 100-150 rpm. The pH of the dissolving liquid was changed at various times to simulate GI transit. At a certain period, 5 ml of samples were changed with fresh 0.1 N HCl. The material was then filtered and measured at 287 nm using a double beam UV-Visible spectrophotometer (UV 3000+, M/s Labindia, Mumbai, India) (36). Male albino rabbits weighing 1.5-2 kg were used for *in vivo* pharmacokinetics. Institutional Animal Ethical Committee (IAEC), Jeeva Life Sciences, Hyderabad, authorized the experiment design protocol (No. IAEC CPCSEA/IAEC/JAS/16/07/21/26).

Grouping and treatment of animals

Eighteen male albino rabbits weighing 1.5-2 kg were selected from the animal house and were divided into three groups of six animals. The first group received pure drug solution via Ryle' tube (8FG). The second group received a solution of optimized solid SNEDDS and the third group received the marketed formulation (Cardivas 3.123 mg).

Dose calculation

The dose for the rabbits was calculated using equation-2

Accelerated stability studies

Stability studies were performed for the optimised solid self-nano emulsifying drug delivery system to evaluate the effects of different storage conditions and different storage durations. The formulation was kept at a constant 40 ± 2°C and 75 ± 5% RH during the study. The cumulative percentage drug release (QT30), emulsification time (minutes) and globule size (nm) of the optimized batch were evaluated for different time intervals.

RESULTS

Mean solubility studies

The Lauroglycol FCC showed the highest solubility of carvedilol (54.66 ± 0.09 µg/mL) among the synthetic lipidic solvents and oils used for equilibrium solubility tests, including Labrafac PG, Labrafil M, Cremophor RH40, and Capmul MCM. Labrafil M had the lowest solubility (9.87 ± 0.04 µg/mL). Figure 1 shows carvedilol solubility in oil. The optimized liquid SNEDDS were dissolved in lauroglycol FCC, yielding a solubility value of 89.25 ± 0.12µg/mL (Supplementary Table S1).

Spectrophotometric estimation of pure drug

Following solvent interference analysis, a calibration curve was plotted at λ_{max} 287 nm and the drug was estimated against 0.1N HCl. The linear correlation and correlation coefficient (R^2 = 0.991) in the concentration of 10-90 µg/mL pure drug range were calculated (Supplementary Table S2) and as shown in Figure 2A and B.

Pseudo-ternary phase diagrams

The phase diagrams of steady nanoemulsion in carvedilol are shown in Figure 3A-E. Tween 20 (surfactant/co-surfactant) (2:1) was chosen for optimal liquid SNEDDS formulation from Lauroglycol FCC (oil) combinations.

Self-emulsification time

The self-emulsification time for the F8 formulation was found to be 3.6 minutes while the F16 was about 60 minutes and the self-emulsification time was more than 40 minutes which usually signifies that the formulation is not optimal and may require adjustments to improve its performance.

$$\text{Total dose (in humans)} \times 0.07 \text{ (factor for each rabbit)} \times 2\text{kg weight of rabbit}/1.5$$
$$= \frac{25 \times 0.07 \times 2}{1.5} = 2.333 \text{ mg}.....\text{equation-2}$$

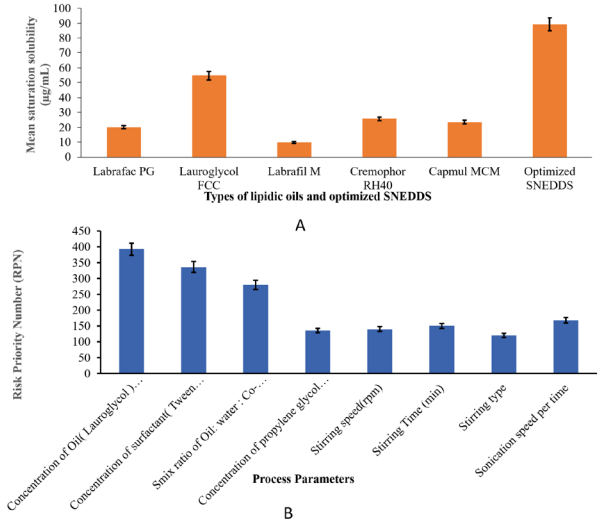


Figure 1. Bar diagram of mean saturation solubility of pure drug in different selected oils (A); Bar diagram of FMEA tool for factors identification (B).

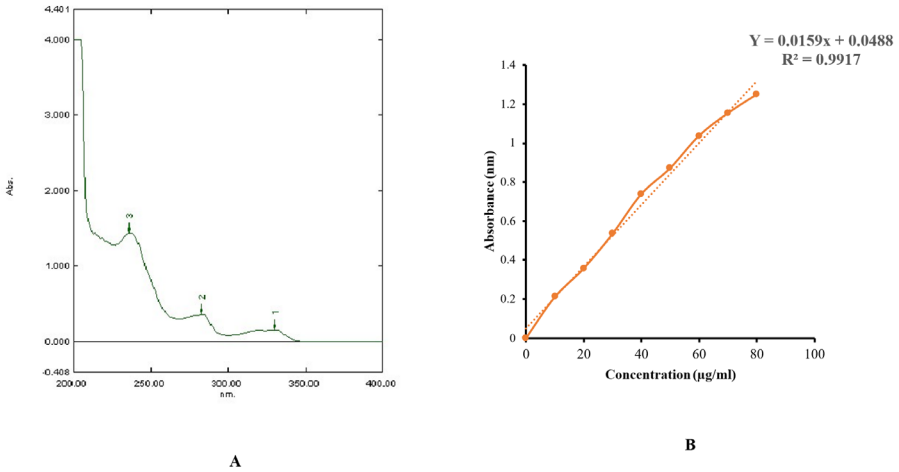


Figure 2. Pure drug concentration (20 µg/mL) spectrum of carvedilol using double beam UV-Visible spectrum (A) and calibration curve of pure drug (carvedilol) in 0.1N HCl solution in different drug concentrations at ¤max 287 nm (B).

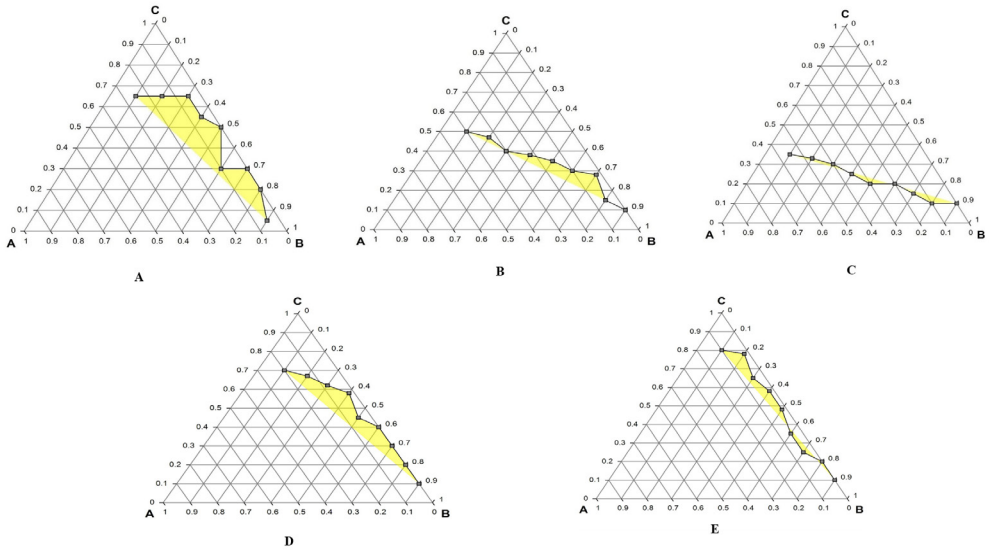


Figure 3. Pseudo ternary phase diagrams of oil: Smix ratio (2:1) (A); Pseudo ternary phase diagrams of oil: Smix ratio (2:2) (B); pseudo ternary phase diagrams of oil: Smix ratio (2:3) (C); Pseudo ternary phase diagrams of oil: Smix ratio (1:1) (D); and Pseudo ternary phase diagrams of oil: Smix ratio (1:2) (E).

Integration of experimental design for statistical optimization

Table 3 shows 17 experimental runs of BBD-based carvedilol-loaded SNEDDS. The model's p-value and f-values were 0.05; supplementary Table S3 shows the model's best fit. Supplementary Table S4 shows the DoE summary for the design. The Box-Behnken model was calculated by fitting it into a mathematical model. The direct impact of the chosen independent parameters, e.g., Lauroglycol FCC concentration (mg/mL) (A), Smix ratio (%v/v) (B), and Tween 20 concentration (mg/mL), on variables including cumulative percentage drug release QT30, ET (min) and globule size (nm) was substantial. Lauroglycol FCC concentration also affected drug release and encapsulation. Published study have shown a favourable link between Lauroglycol FCC concentration, globule size, and drug release. Lipophilic drugs breakdown better in lipid, improving drug release. As pure drug is lipophilic, it dissolves quickly in Lauroglycol FCC, showing that entrapment efficiency is primarily affected by FCC concentration. The 2D and 3D plots in Figure 4 show that formulation 8 had the highest drug release and desirable globule size. Figure 4A-F and Figure 5A-F show perturbation and predicted vs. actual graphs. Smix ratio concentration synergistically solubilizes lipophilic pharmaceuticals, improving SNEED entrapment and drug release (39).

Quadratic polynomial equations analysis

The quadratic polynomial equations from the mathematical model for individual responses are as follows:

Cumulative percentage drug release (QT30) =+58.99-24.836*A-10.78*B-7.52*C-6.09*AB-18.47 *AC+3.9*BC+1.06*A2-3.69*B2+13.72*C2...equation-3

Table 3. Different formulation composition of carvedilol loaded SNEDDS of obtained seventeen experimental runs as per BBD along with the obtained CQAs responses, coded and actual levels

Run	Factor 1 A: Lauroglycol FCC (X1) mg	Factor 2 B: Smix (X2) mL	Factor 3 C: Tween 20 (X3) mg	Response 1 QT30 (Y1) %	Response 2 ET (Y2) min	Response 3 Globule size (Y3) nm
1	0	0	0	52.12	57.00	289.65
2	0	-1	1	83.25	33.00	220.15
3	1	-1	0	34.12	58.90	293.45
4	-1	0	-1	88.25	25.00	189.22
5	0	-1	-1	86.22	30.00	202.56
6	-1	0	1	90.23	10.20	130.56
7	0	0	0	67.25	45.00	256.25
8	1	0	-1	94.25	3.60	120.55
9	1	0	1	22.36	59.00	301.25
10	-1	1	0	90.80	15.00	152.65
11	0	0	0	54.36	55.00	280.15
12	-1	-1	0	90.33	8.00	105.26
13	0	0	0	60.21	52.00	270.33
14	0	0	0	61.02	49.00	268.10
15	0	1	1	59.66	53.00	306.54
16	1	1	0	10.22	60.00	307.90
17	0	1	-1	46.97	58.00	290.34

Independent Variables	Coded and actual levels		
	Low (−1)	Medium (0)	High (+1)
A: Lauroglycol FCC (mg)	75	100	125
B: Smix (mL)	150	200	250
C: Tween 20 (mg)	150	200	250

BBD, Box-Behken Design, CQAs, critical quality attributes; FCC, food chemicals codex; QT30: cumulative percentage drug release at 30 minutes; ET, emulsification time

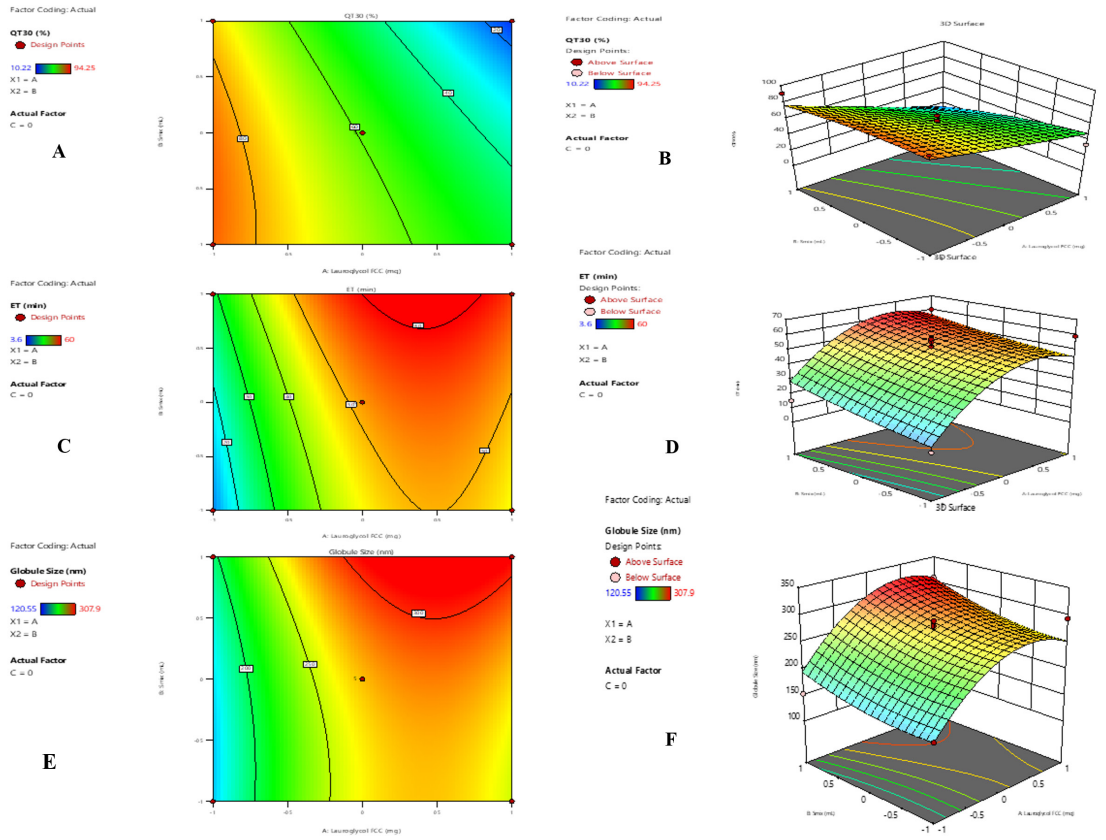


Figure 4. Contour (2D) and response surface (3D) plots of specific independent factors on specific desired dependent responses, such as cumulative percentage of drug release (QT30) (A) and (B); emulsification time (minutes) (C) and (D); globule size (nm) (E) and (F).

Self emulsification time (ET) (min)

=+51.60+15.41*A+7.01*B+4.83*C-1.47*AB+17.55*AC-2.00*BC-17.59*A2+1.46*B2-9.56*C2.....equation-4

Globule size(nm)

=+272.90+51.35*A-22.63*B+16.95*C+5.50*AB+59.78*AC-0.375*BC-56.89*A2+12.55*B2-30.55*C2.....equation-5

2D and 3D response surface interpretations

Effect of the factor on cumulative % drug release (QT30)

The contour plot and 3D plot for CQA cumulative drug release QT30 (min) are shown in Figure 4A and B. The formulation with the highest drug release, testing run 8, with 94.25%. The run 16 minimum of 10.22% indicates above 90.00% drug release in 30 minutes.

Effect of the factor on self-emulsification time (ET)

The contour and 3D CQA emulsification time plots are shown in Figure 4C and D. Trial run No. 8 had the lowest value according to these formulae.

Effect of factors on globule size

The CQA globule size contour and 3D plots are shown in Figure 4E and F. The formulation with the lowest vesicle size, 120.55 nm, was trial run 8. Run 16 had the largest vesicle, 307.9 nm.

Overlay plots analysis

The overlay plot indicated that 25 mg drug, 125mg/mL Lauroglycol FCC and 200mg/mL Smix was the optimum composition of SNEDDS. Supplementary Table S5 summarizes the optimization method and shows experimental and expected formulation responses indicating where adjusting independent factor concentrations maximized dependent variable results. The graph displays multiple passes that produced optimal factor values to guarantee the simultaneous viability of every operational constraint. Process and formulation restrictions are displayed by overlay graphs (Figure 6).

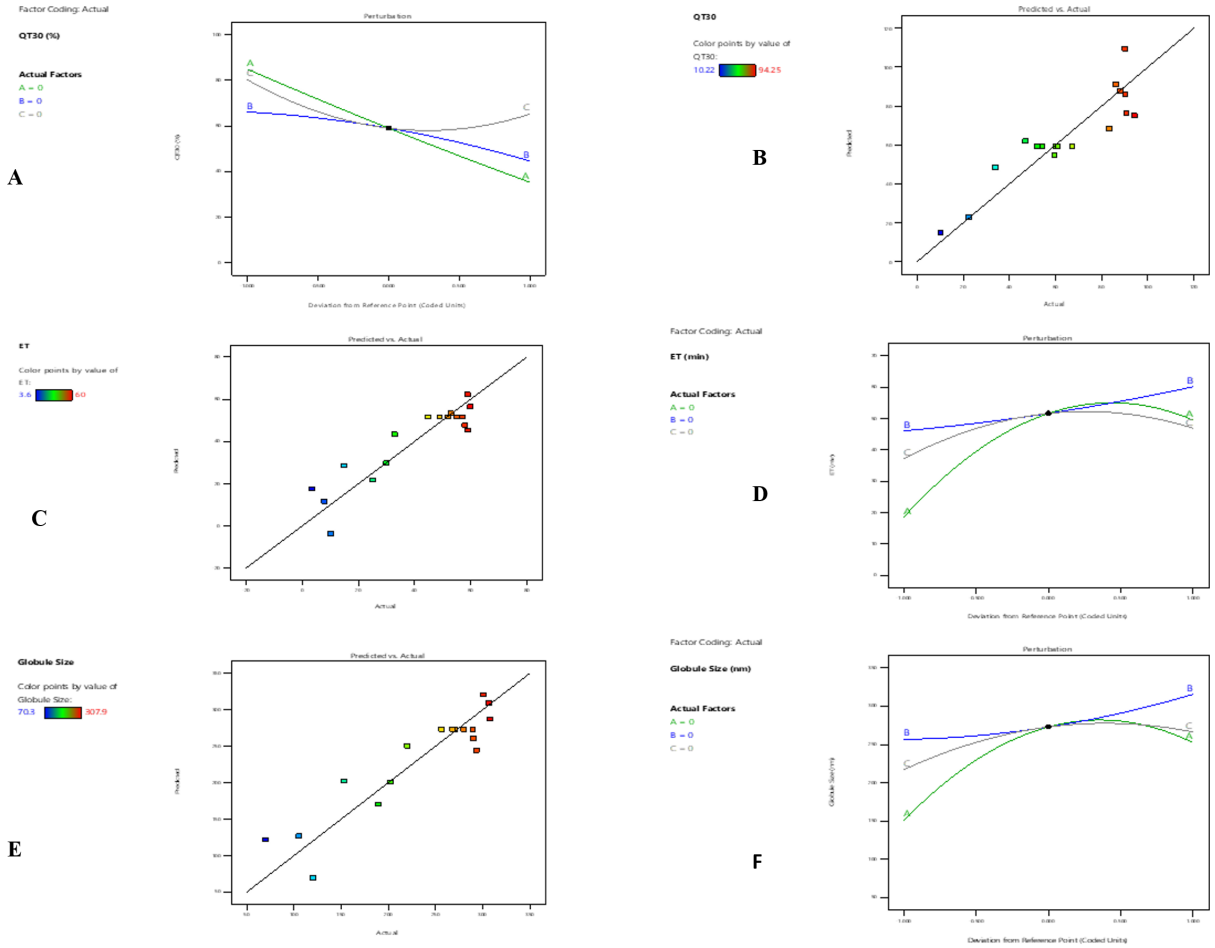


Figure 5. Predicted, actual and perturbation plots of selected independent factors on selected dependent factors cumulative percentage of drug release (QT30) (A) and (B); emulsification time (minutes) (C) and (D); globule size (nm) (E) and (F).

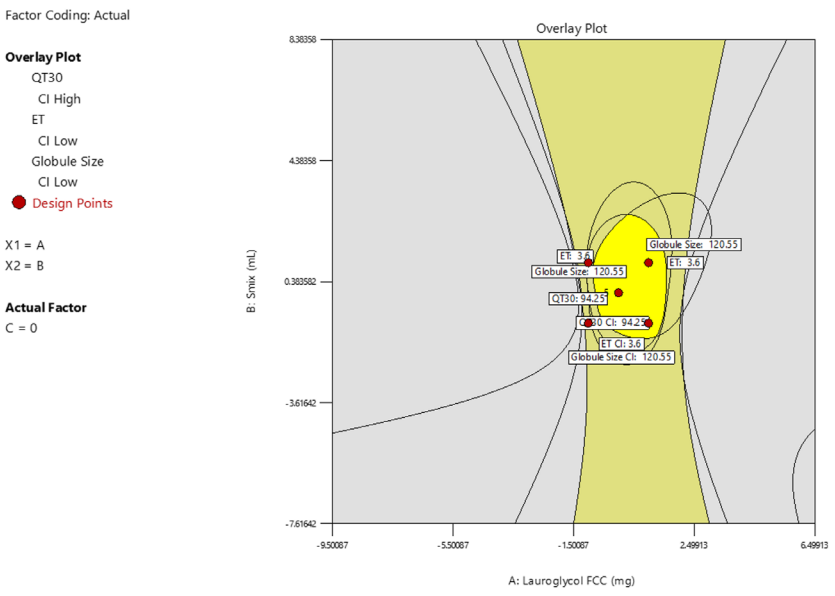


Figure 6. Overlay plot for the identification of the design space

Characterization of solid SNEDDS studies

FT-IR studies

Figure 7A and B compare the FT-IR spectra of optimized SNEDDS (F8) to pure drug. Pure drug is characterized by amine (3,102 cm^{-1}) tertiary, C = O (1,083 cm^{-1}), C–N (1269 cm^{-1}, 11,266 cm^{-1}), C–S (630 cm^{-1}), and N–H (3,372 cm^{-1}) bands.

DSC studies

The DSC thermograms for the pure drug showed an endothermic peak at 161.6°C with onset and end set temperatures of 157.0°C and 173.9°C, respectively. F8 formulation showed a strong endothermic peak at 160.02°C, with start and end set temperatures ranging from 155.37°C to 163.66°C. These findings indicated no interactions between pure drug and other formulation excipients (Figure 8A and B).

Zeta potential, viscosity, droplet size analysis, and encapsulation efficiency

In the context of colloidal dispersion, the zeta potential offers insight into its prospective stability. Dispersion stability and aggregation resistance are achieved when charged particles with higher positive or negative zeta potential reject one other. The optimized SNEDDS had a zeta potential of -19.6 mV (Figure 9A). The viscosity of the optimized liquid SNEDDS formulation was found to be 0.397 cP (Figure 9B). Droplet size affects oral emulsion storage and *in vivo* stability, making it a key SNEDDS evaluation criterion. Furthermore, droplet size greatly impacts medication release and absorption. The improved SNEDDS formulation had a droplet size of 120.55 nm (Figure 9C). As demonstrated in Figure 9D, the optimized drug loaded SNEDDS had 90.45% encapsulation

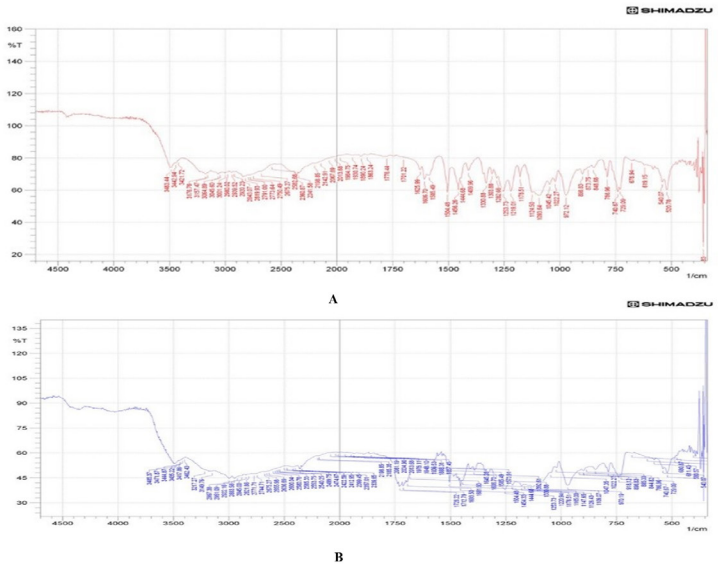


Figure 7. FT-IR spectrum of carvedilol (A) and an optimized formulation batch of carvedilol-loaded SNEDDS (B).

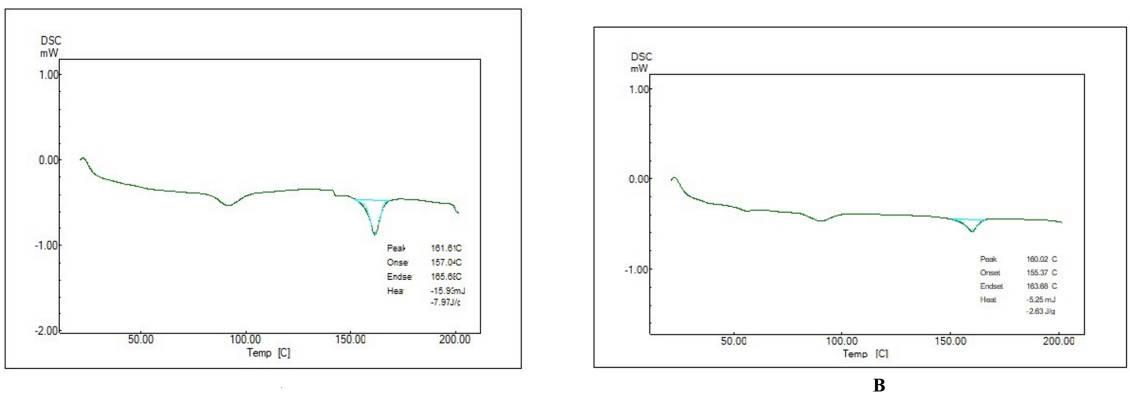


Figure 8. DSC thermograms of pure drug (A), and optimized formulation batch of carvedilol-loaded SNEDDS (B)

efficiency, which is superior to the other formulations. The globule size picture of an optimized formulation batch of carvedilol-loaded SNEDDS and the zeta potential picture of an optimized formulation batch of carvedilol-loaded SNEDDS are depicted in Figure 10A and B. The characterization data of seventeen different formulations of carvedilol-loaded SNEDDS are shown in Table 4.

Powder X-ray diffraction

The pure drug and optimized SNEDDS powder X-ray diffraction are exhibited in Figure 11A and B. Pure drug exhibited prominent peaks at 6.9°, 18.3°, 19.9°, 25.6°, 21.3°, 25.2°, and 26.4°, suggesting a crystalline structure. The minimum peak intensity at certain angles decreased gradually in the optimized formulation batch, suggesting an amorphous structure.

Table 4. Characterization data of different seventeen formulations of carvedilol loaded self-nanoemulsifying drug delivery systems

Formulation code	Zeta potential (mv)	Viscosity (cP)	Globule Size (nm)	Encapsulation efficiency (%)
F1	-3.4±0.29	0.369±0.45	289.65±0.22	47.36±0.04
F2	-2.6±0.69	0.325±0.33	220.15±0.21	36.50±0.20
F3	-9.6±0.03	0.258±0.25	293.45±0.025	75.55±0.42
F4	-5.4±0.26	0.297±0.64	189.22±0.40	68.22±0.32
F5	-1.4±0.09	0.345±0.02	202.56±0.55	72.14±0.62
F6	-3.9±0.66	0.224±0.09	130.8±0.06	82.10±0.72
F7	-6.5±0.36	0.129±0.53	256.25±0.01	88.65±0.32
F8	-19.6±0.90	0.397±0.04	20.55±0.02	90.45±0.12
F9	-5.9±0.06	0.373±0.33	301.25±0.46	86.74±0.06
F10	-2.6±0.03	0.459±0.64	152.65±0.06	75.50±0.08
F11	-2.3±0.09	0.449±0.96	280.15±0.22	69.57±0.55
F12	-4.2±0.26	0.423±0.33	105.26±0.35	65.74±0.03
F13	-2.3±0.22	0.290±0.02	270.33±0.65	58.22±0.09
F14	-5.6±0.03	0.122±0.06	268.1±0.77	56.10±0.33
F15	-4.3±0.09	0.154±0.07	306.54±0.02	49.36±0.08
F16	-6.2±0.26	0.245±0.05	307.9±0.06	80.22±0.26
F17	-7.3±0.22	0.289±0.48	290.34±0.09	49.27±0.23

n: No. of observations (n = 3)

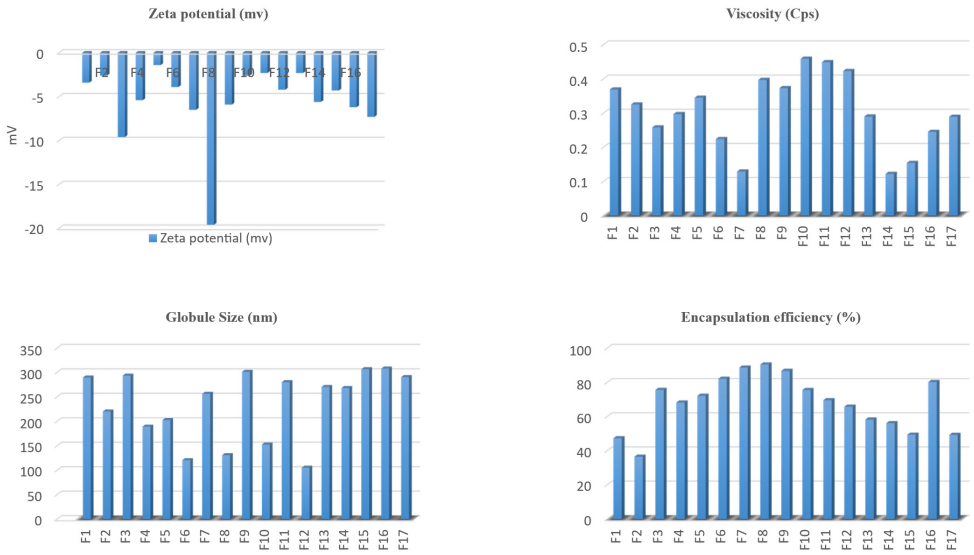


Figure 9. Bar graph representation of characterization of data including zeta potential (A), viscosity (B), globule size (C), and encapsulation efficiency (D) of seventeen formulation batches of carvedilol-loaded SNEDDS

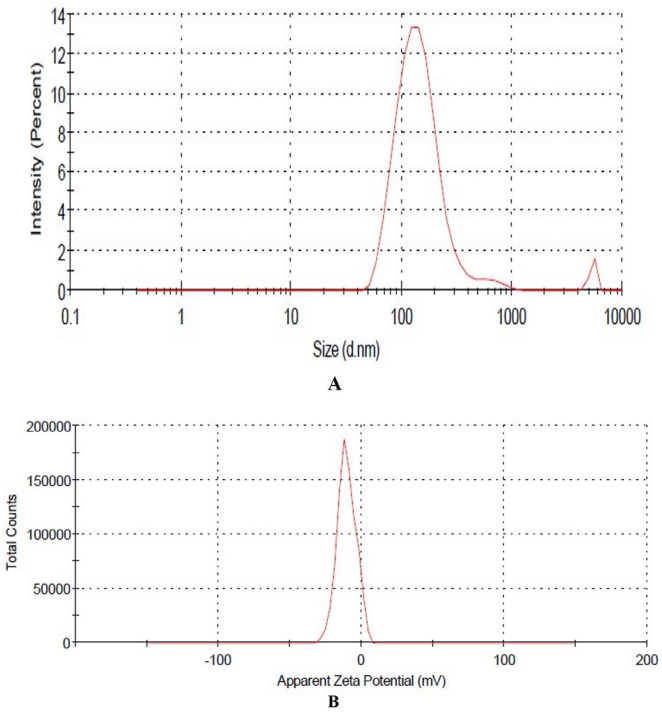


Figure 10. Globule size picture of optimized formulation batches of carvedilol-loaded SNEDDS (A) and zeta potential picture of optimized formulation batches of carvedilol-loaded SNEDDS (B)

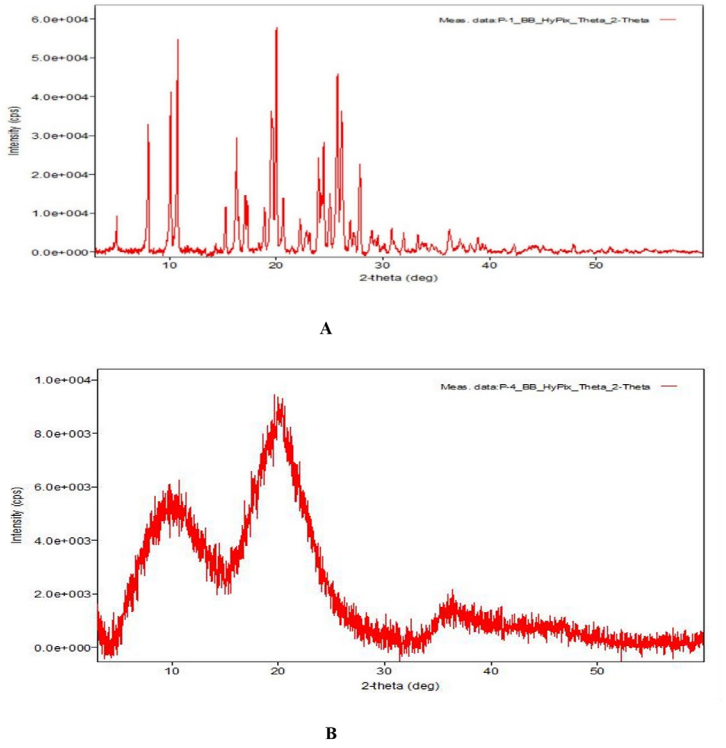


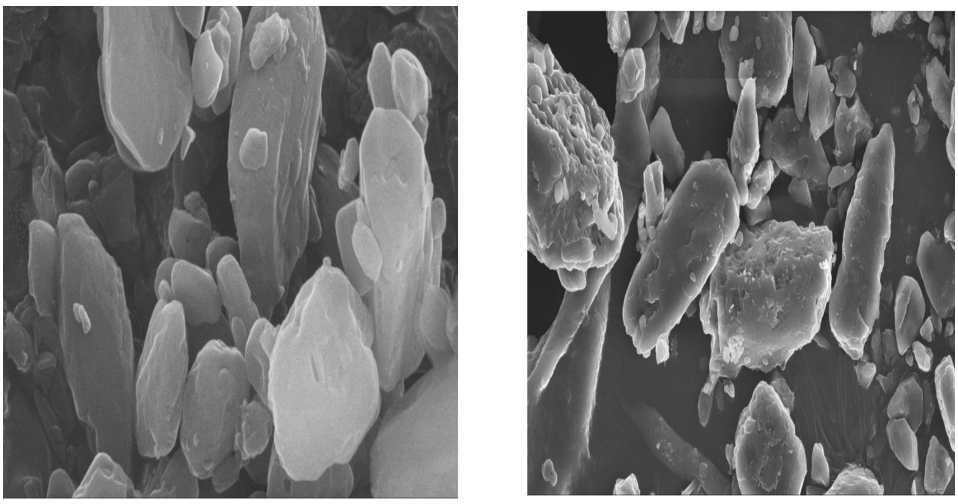
Figure 11. P-XRD curves of pure drug (A) and an optimized formulation batch of carvedilol-loaded SNEDDS (B)

Scanning electron microscopy

Based on morphology, SEM was used to study pure drug and optimized SNEDDS. SEM micrographs Figure 12A and B show smooth, spherical drug-loaded self-nano emulsifying DDS (F8).

In vitro drug release study

The drug release behaviour of pure-drug and optimized SNEDDS is shown in Figure 13. The graph shows that the optimized batch released 94.25% more drug at 30 minutes than the pure



A B

Figure 12. SEM images of pure drug (A) and an optimized formulation batch of carvedilol-loaded SNEDDS (B)

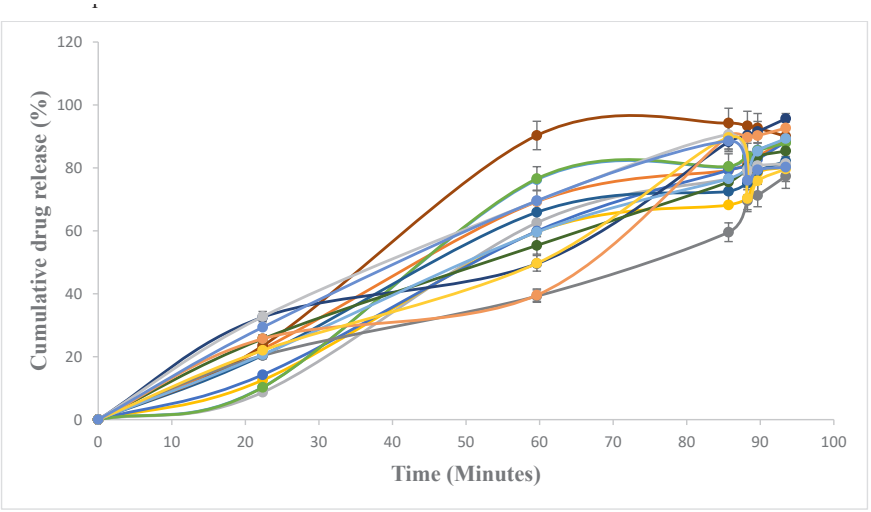


Figure 13. *In vitro* drug release curve of seventeen different formulation batches of carvedilol-loaded SNEDDS

Table 5. *In vivo* pharmacokinetic data of the pure drug and optimized formulation batch (F8) of carvedilol loaded self-nanoemulsifying drug delivery systems

Formulations	C_{max} (ng/mL)	T_{max} (h)	K^e	AUC_0^∞ (µg/h/mL)
Pure drug (Carvedilol)	822.69	1 hour	155.36	12.650
Optimized SNEDDS (F8)	2,563.23	1 hour	182.7	33.382
Marketed tablet formulation (cardivas)	1,089.33	40 minutes	165.96	15.690

drug (Supplementary Table S6). Ideal drug-loaded SNEDDS R^2 values were 0.899, 0.978, and 0.998 for pure drug, 0.981 for zero-order, 0.992 for first-order, and 0.934 for the Higuchi model. The Higuchi model for pure-drug and optimized SNEDDS matched best according to the multiple kinetic models' R^2. Use of such models established R^2 values for pure drug and SNEDDS kinetic models separately. Thus, pure drug release follows Fickian diffusion kinetics, whereas optimized SNEDDS follows non-Fickian kinetics.

In-vivo pharmacokinetic study

The mean serum concentration of drug vs. time plots for the pure drug, optimized SNEDDS,

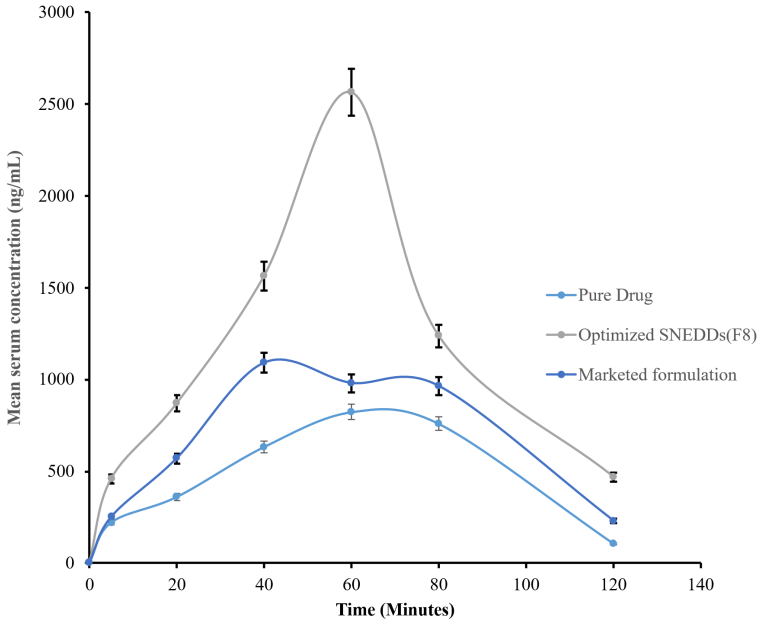


Figure 14. Mean serum drug concentration (ng/mL) vs the time (minutes) curve of pure drug, the optimized formulation batch of carvedilol-loaded SNEDDS and the marketed tablet formulation (cardivas)

Table 6. Accelerated stability conditions data of selected parameters for optimized formulation batch of carvedilol loaded SNEDDS at 40°C ± 2°C and 75 ± 5% RH

Time (months)	Cumulative drug release at QT30 (%)	Emulsification time (minute)	Vesicle size (nm)
0	94.25±0.69	3.6±0.28	130.8±0.53
1	92.66±0.77	5.30±0.98	135.22±0.60
2	90.22±0.99	6.54±0.24	144.98±0.03
3	89.69±0.63	7.98±0.33	149.36±0.09
6	88.27±0.25	9.75±0.64	158.45±0.07
p-value α ≤ (0.05) significant difference exists	0.065	0.070	0.058

n: No. of observations (n = 3)
QT30, cumulative percentage drug release at 30 minutes

and the marketed formulation showed improvement of oral bioavailability of the final selected batch compared to the pure drug and marketed product (Figure 14). The estimated difference *in vivo* pharmacokinetic parameters for the pure drug, optimized SNEDDS, and marketed formulation are depicted in Table 5. The optimized formulation showed a Cmax of 2,563.23 ng/mL, Tmax of 1 h, and AUC0∞ of 33.382 µg/h/mL, whereas AUC0∞ of pure drug 12.650 µg/h/mL.

Accelerated stability study

The *p*-value for all CQAs was larger than 0.05, indicating no statistically significant change. Since the CQAs did not alter throughout the investigation, the optimized SNEDDS loaded with carvedilol satisfied stability requirements based on the stability study (Table 6).

DISCUSSION

The mean saturation solubility measured with different lipidic solvents clearly indicates that lauroglycol FCC exhibited the highest solubility due to its lipophilic nature and the log P value of 4.2 of the carvedilols. Thus, this drug is an ideal candidate for the development of SNEEDS with high permeability and a higher volume of distribution. The UV spectrophotometric estimation clearly indicates that the drug was quantified spectrophotometrically against 0.1N HCl with wavelength maxima at λ_{max} 287 nm, within the linearity range of (10-90 µg/mL) and R^2 value of

0.991 which clearly indicates that it satisfies the Beers Lambert Law. The mapping of the nanoemulsion region was effectively carried out using the pseudo ternary phase diagram approach which assisted in the selection of the optimized and most judicious combination of the oil and surfactants in the preferred ratio. Hence a ratio of (2:1) of (Lauroglycol FCC: Tween 20) was selected as an optimal region for development of optimized SNNEDS with improved oral bioavailability (39). The self-emulsification time of less than 5 minutes suggests that the SNEDDS formulation is both efficient and effective in delivering the active pharmaceutical ingredient, ensuring rapid and complete dispersion, consistent drug absorption, and improved bioavailability. The self-emulsification time for the F8 formulation was found to be 3.6 minutes, which is Grade D as per USP protocol for emulsification time. The experimental design for statistical optimization was carried out based on the BBD model and clearly indicates that the observed responses were as per the pre-determined target fixed for the QTPP. According to simulations of cumulative drug release QT30 (min), this reddish zone is a high Laurogylocol FCC (1) and a moderate Smix ratio concentration (0). The blue zone with the least drug release was seen at high Laurogylocol FCC and Smix ratio concentrations (+1). ET values were greatest in the blue zone, which is predicted to be predominant at high Laurogylocol FCC levels (+1) and moderate Smix ratio concentrations (0). Run number 16 had the highest ET value at 60 min. Higher up, the pale reddish zone was seen (+1). The simulations of globule size exhibited a dark blue zone showing the smallest vesicle size and is predicted to be prominent with high Laurogylocol FCC (+1) and moderate Smix ratio (0) concentrations. At greater (-1) concentrations, the bright red zone had the largest vesicles. The F8 formulation had identical intensity peaks, and the physical mixture combinations had additional intensity peaks. No interactions between the pure medication and excipients were found. That no new peaks were found clearly indicates that there was no chemical reaction and that stability of the drug was well maintained. No degradation peaks were noticed. The DSC thermograms clearly show that there are no interactions between the pure drug and optimized SNEEDS (F8) which indicates that the drug is molecularly dispersed (amorphous or solubilized state) in the lipid matrix. That indicates complete solubilisation in the nano emulsion system (40). The optimized SNEDDS (F8) showed a zeta potential of –19.6 mV, indicating a moderately negative surface charge. Although the formulation used non-ionic surfactants, negative potential may arise from ionization of oil or drug at the interface. The magnitude of the zeta potential suggests sufficient repulsive forces to maintain colloidal stability and prevent coalescence of droplets, thereby supporting the long-term stability of the nanoemulsion. The viscosity of the optimized carvedilol SNEDDS was found to be 0.397 cP, which is suitable for oral administration and supports rapid self-emulsification upon aqueous dilution. The low viscosity ensures proper flow behaviour during processing and contributes to the formulation's spontaneous dispersion and uniform nanoemulsion formation. The mean droplet size of 120.55 nm with a narrow size distribution (PDI = 0.38) confirms the formation of a uniform and stable nanoemulsion upon aqueous dilution. The small droplet size contributes to enhanced surface area for absorption, ensuring improved oral bioavailability of carvedilol. The optimized carvedilol SNEDDS formulation exhibited an entrapment efficiency of 90.45 ± 0.12%, indicating that the drug was effectively solubilized within the lipid-based system. The high entrapment suggests good compatibility and solubilization capacity of the selected oil, surfactant, and co-surfactant. This is expected to enhance the drug's oral bioavailability by maintaining it in a solubilized form after administration. The optimized SNEDDS formulation showed no characteristic diffraction peaks of carvedilol, indicating that the drug was completely solubilized and existed in an amorphous state within the formulation (41). This transition from crystalline to amorphous form is expected to enhance the dissolution and oral bioavailability of carvedilol. The SEM images of pure carvedilol displayed distinct crystalline structures with sharp edges, confirming its crystalline nature. In contrast, SEM micrographs of the optimized solid SNEDDS showed an amorphous, porous morphology with no visible crystalline drug particles. The *in vitro* release study showed that the optimized SNEDDS of carvedilol released 94.25%

of the drug within 30 minutes. The formulation demonstrated rapid self-emulsification and formed a clear nanoemulsion with a droplet size of 120.55 nm. No drug precipitation was observed upon dilution, indicating excellent solubilization stability (42). These results confirm the potential of SNEDDS in enhancing the dissolution and, hence, the oral bioavailability of carvedilol. The *in vivo* studies indicate that the optimized batch had three times greater bioavailability than the pure drug. This might be due to drug solubility, increased gastrointestinal membrane absorption and drug penetration. The *p*-values for the concerned CQAs had no significant changes in the observed responses which clearly shows that the optimized SNEEDS of carvedilol were stable under the accelerated conditions for 6 months.

CONCLUSION

By using QbD, this study prepared a carvedilol-loaded self-nano emulsifying drug delivery system with increased bioavailability. The study found that QbD helped identify and optimize processes and material variables to achieve product quality goals. High concentrations of Lauroglycol FCC and intermediate concentrations of Smix ratio are the primary CMAs attributes that significantly affect cumulative drug release (QT30), ET (minutes), and globule size (nm), making it easier to develop stable solid SNEDDS of carvedilol with improved therapeutic benefits. The cumulative percent drug release QT30 (min) was more than 90.00%, indicating that this route provides better drug release. The nanoemuslion size, as shown by SEM and particle size analysis, confirms that it can be effectively absorbed by the body. The lower peak intensities in p-XRD analysis shows that the formulation is amorphous compared to the pure drug. The ICH stability investigations likewise showed no significant changes in the research parameters. Thus, this unique approach may be used to construct stable carvedilol SNEDDS for acute hypertension therapy.

ACKNOWLEDGMENTS

The authors are highly thankful to Amity Institute of Pharmacy, Amity University, Kolkata for providing the facility to carry out the research study.

FUNDING

There is no funding associated with this research study.

CONFLICTS OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ADDITIONAL INFORMATION

Authors' contributions

R.L.M.: Conducted the initial experimental investigations pertaining to formulation and characterisation; S.S.: Original draft, supervising, writing, formal analysis, software applications, conceptualisation, and methodology; S.K.M.: DG and B.R.J.: Verified writing, writing correct analysis, software applications typographical and grammatical errors, etc., in accordance with journal rules. The paper has been read and approved by all authors.

Data availability

All necessary data during this research study are included in this article.

Supplementary materials

The following supporting information can be downloaded at: Supplementary file

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Supplementary

Table 1. Mean saturation solubility data of pure drug in different selected oils and optimized batch of carvedilol loaded SNEDDS

Selected oils and optimised SNEDDS	Mean saturation solubility (µg/mL)
Labrafac PG	20.15±0.06
Lauroglycol FCC	54.66±0.09
Labrafil M	9.87±0.04
Cremophor RH40	25.66±0.08
Capmul MCM	23.4±0.05
Optimized SNEDDS	89.25±0.12

N: Number of observations (n=3)

Supplementary Table 2. Calibration curve data of pure drug in different concentrations (µg/ml) v/s absorbance in 0.1N HCl solution at λ_{max} 287 nm

Concentration (µg/mL)	Absorbance (nm)
0	0
10	0.102
20	0.221
30	0.326
40	0.465
50	0.569
60	0.654
70	0.789
80	0.965

Supplementary Table 3. Summary of ANOVA for different factors and its significance with respect to quadratic model

Source	Cumulative drug release QT30 (%)		ET (Minutes)		Globule size (nm)	
	F-value	**p-value**	**F-value**	**p-value**	**F-value**	**p-value**
Model	3.71	0.0489*	3.88	0.0438*	4.48	0.0303*
A: Lauroglycol FCC (mg)	18.86	0.0034	12.05	0.0104	10.12	0.0155
B: S_{mix} (mL)	3.56	0.1013	2.50	0.1582	3.43	0.1063
C: Tween 20 (mg)	1.73	0.2297	1.18	0.3131	2.37	0.1676
AB	0.5677	0.4758	0.0552	0.8210	0.1337	0.7254
AC	5.22	0.0563*	7.81	0.0267*	11.07	0.0127*
BC	0.2344	0.6431	0.1015	0.7593	0.0002*	0.9881
A^2	0.0181	0.8967	8.26	0.0238*	10.56	0.0141*
B^2	0.2187	0.6542	0.0571	0.8179	0.3641	0.5653
C^2	3.03	0.1253	2.44	0.1621	2.03	0.1976
Lack of fit	15.80	0.0111*	14.80	0.0124*	28.25	0.0038*

*Significant levels: less than α value (0.05); ET: Self-emulsification time

Supplementary Table 4. Summary of design of experiment with various parameters fitting to quadratic model

Responses	Cumulative drug release QT30 (%)	ET (min)	Globule size (nm)
R^2	0.8267	0.8330	0.75879
Adjusted R^2	0.6038	0.6182	0.66548
Predicted R^2	0.5788	0.4732	0.69325
Adeq Precision	7.6501	6.8458	4.9658
Std. Dev.	0.8267	0.8330	6.253

ET: Self-emulsification time; R^2: Regression correlation coefficient; Std. Dev: Standard deviation

Supplementary Table 5. Constraints for the process of optimization of carvedilol loaded SNEDDS using DoE

Run 8 Response	Predicted Mean	Predicted Median	Observed	Std Dev	SE Mean	95 % CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
QT30 (%)	74.931	74.931	94.25	16.172	14.005	41.812	108.050	-26.469	176.332
ET (Minutes)	17.487	17.487	3.6	12.556	10.873	-8.225	43.200	-61.237	96.212
Globule Size (nm)	121.569	121.569	130.8	45.035	39.002	29.343	213.794	-160.801	403.938

Supplementary Table 6. In vitro cumulative percentage drug release data of pure drug, and prepared suggested seventeen formulations of carvedilol-loaded SNEDDS

Time (Minute)	Cumulative drug release (%)																
	Pure drug	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	22.36±0.02	10.32±0.05	22.44±0.08	8.69±0.45	12.56±0.05	14.25±0.04	10.23±0.25	20.45±0.05	23.45±0.15	20.36±0.44	25.99±0.04	32.55±0.04	25.6±0.07	20.65±0.10	25.69±0.01	32.74±0.05	22.01±0.02	29.35±0.01
20	59.63±0.06	76.23±0.24	69.25±0.75	62.56±0.05	59.65±0.25	59.88±0.02	76.56±0.15	65.89±0.25	90.3±0.25	39.25±0.57	43.25±0.07	49.56±0.07	55.36±0.06	59.58±0.11	39.56±0.04	69.45±0.25	49.67±0.04	69.55±0.04
30	85.69±0.60	80.26±0.20	79.25±0.25	76.56±0.02	68.22±0.35	79.26±0.03	80.45±0.25	72.56±0.02	94.25±0.03	59.56±0.08	75.22±0.04	88.26±0.04	75.52±0.08	76.55±0.04	90.22±0.06	90.57±0.01	89.63±0.08	88.56±0.07
40	88.25±0.55	83.22	81.22±0.15	78.57±0.09	70.28±0.55	80.45±0.05	83.64±0.35	75.12±0.04	93.33±0.04	69.58±0.04	76.25±0.02	90.36±0.07	80.25±0.05	79.63±0.07	89.69±0.22	79.25±0.04	70.24±0.57	76.02±0.08

		±0.02															
50	89.64±0.02	85.69±0.03	83.65±0.45	79.01±0.12	78.36±0.03	82.69±0.22	85.47±0.45	78.95±0.08	92.65±0.05	71.22±0.06	78.95±0.10	91.54±0.08	83.66±0.04	85.26±0.08	90.25±0.30	80.69±0.05	75.89±0.35	79.36±0.04
60	93.47±0.08	88.9±0.42	88.64±0.03	81.04±0.36	80.25±0.04	88.25±0.15	87.9±0.25	82.12±0.09	90.02±0.70	77.36±0.04	80.66±0.14	95.625±0.03	85.36±0.07	89.33±0.01	92.65±0.80	81.36±0.09	79.66±0.67	80.22±0.09

N: Number of observations (n=3)