Ind. J. Pharm. Edu. Res., 2024; 58(1s):s126-s138.
https://www.ijper.org

Original Article

Design Expert-Implemented Nimodipine-Loaded Lyophilized Nanoemulsifying Drug Delivery System for Improved Oral Bioavailability and Physical Stability

Prativa Das, Jnyanaranjan Panda, Goutam Kumar Jena*, Chinam Niranjan Patra, Kahnu Charan Panigrahi

Department of Pharmaceutics, Roland Institute of Pharmaceutical Sciences, Khodasingi, Berhampur, Ganjam, Odisha, INDIA.

ABSTRACT

Purpose: To improve the oral bioavailability as well as the physical stability of nimodipine, Design-Expert-driven nimodipine loaded nanoemulsifying drug delivery system was developed with a certain quality target product profile. **Materials and Methods:** In this investigation, the three components triacetin as oil phase, labrasol as a surfactant, and plurol oleique CC 497 as co-surfactant were selected after screening. The ratio of surfactant and co-surfactant (S_{mix}) was selected from the pseudo-ternary phase diagram drawn by using ProSim ternary software. A d-optimal mixture design was employed to optimize the formulation. The dynamic light scattering, Fourier transform Infrared, Differential Scanning Calorimetry, X-ray Diffraction, Scanning Electron Microscopy, *in vitro* drug release, stability study, and *in vivo* pharmacokinetic studies were carried out for the characterization of the optimized formulation. **Results:** The globule size, PDI, and Zeta potential of the optimized formulation were found to be 322.1 nm, 0.48, and -14.5 mV respectively. The result of *in vivo* pharmacokinetic studies exhibited three-fold enhanced oral bioavailability of the optimized nanoemulsion as compared to the pure drug of nimodipine and the physical stability of the optimized nanoemulsion improved significantly as compared to the pure drug. **Conclusion:** The NIMO-loaded nanoemulsifying drug delivery system can be successfully fabricated by implementing the Design-Expert with improved oral bioavailability and physical stability significantly as compared to the pure drug of NIMO.

Keywords: Bioavailability, Physical stability, Pseudo ternary phase diagram, Design-Expert, Lyophilized, Nanoemulsifying drug delivery system.

Correspondence:
Dr. Goutam Kumar Jena
Department of Pharmaceutics, Roland Institute of Pharmaceutical Sciences, Khodasingi, Berhampur, Ganjam, Odisha- 760010, INDIA.
Email: goutam2902@gmail.com

Received: 25-01-2023;
Revised: 22-03-2023;
Accepted: 13-08-2023.

INTRODUCTION

Nimodipine (NIMO) is chemically 1,4-dihydropyridine. It is a second-generation calcium channel blocker that is recommended especially for the treatment of cerebral vasospasm after subarachnoid hemorrhage, prophylaxis, and management of hypertension and stroke.[1] Due to low solubility and high permeability, NIMO has been placed under BCS class II.[2] It is reported to have $t_{1/2}$ of 7-8 hr and oral bioavailability of 13% approximately.[3] Such a low oral bioavailability in healthy human volunteers can be explained by its low aqueous solubility and considerable hepatic metabolism. Due to less $t_{1/2}$ of NIMO, frequent administration of the drug is required and that leads to patient incompliance and inconvenience.[1] The injectable form of NIMO as an alternative to oral administration is also reported with the enhancement of oral bioavailability, but its use is again limited due to its safety issues and patient incompliance.

Hence developing an oral dosage form of NIMO is a great challenge. To encounter the challenges associated with NIMO as mentioned above, NIMO nanoemulsion was developed in the current research.In the last few decades, lipid-based nanoformulations have been used as the most potential solubility enhancement technique for poorly aqueous soluble drugs. The most popular lipid-based dosage forms are nanoemulsion, Solid Lipid Nanoparticles (SLN), Nanostructured Lipid Carriers (NLC), Self-Emulsifying Drug Delivery Systems (SEDDS)[4-6] Self Nanoemulsifying Drug Delivery Systems (SNEDDS), liposomes, etc. which can significantly enhance solubility and bioavailability of poorly water-soluble drugs.[7-9] In all these lipid-based nanocarriers, the solubilization performance of poorly water-soluble drugs is improved due to their increased surface areas, which leads to enhancing their oral bioavailability.[10-13]

A revolutionary change has been noticed since the implementation of Quality by Design (QbD) as a mandatory requirement of the USFDA for the development of dosage

DOI: 10.5530/ijper.58.1s.12

forms to build quality into the product in the pharmaceutical industry.[4,14,15] Quality by a testing concept has disappeared after the implementation of the concept of QbD as quality is built into the product. In the current research, the concept of QbD has been implemented to predict the potential impacts of Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) as independent variables on Critical Quality Attributes (CQAs) as dependent variables of NIMO nanoemulsion to obtain desired Quality Target Product Profile (QTPP).[16,17] Shambhavi Dhananjay Acharya *et al.*, 2020 fabricated a QbD-driven curcumin-loaded nanoemulsion and reported a thermodynamically stable nanoemulsion with more cytotoxic potential as compared to pure drug using HT29 cell lines.

The current research has been focused on developing NIMO-loaded nanoemulsion employing the QbD approach by taking three components triacetin as oil, labrasol as the surfactant, and plurol oleique CC 497 as co-surfactant, respectively to improve oral bioavailability and stability of NIMO. A d-optimal mixture design-implemented formulation using the above combination of oil, surfactant, and co-surfactant is the first time reported in our current investigation and found to produce stable and effective nanoemulsion. The high-pressure homogenization technique and sonication technique were employed to prepare the NIMO nanoemulsion. A d-optimal mixture design has been adopted to investigate the potential impact of % of oil, % of S_{mix} (Mixture of surfactant and co-surfactant), and % of water as independent variables on the Globule Size (GS), Percentage cumulative drug release in 15 min (Q15), and Polydispersity Index (PDI) as dependent variables of NIMO nanoemulsion.

MATERIALS AND METHODS

Materials

Nimodipine had been obtained from Ms. RA Chem Pvt. Ltd., Hyderabad, India as a gift sample. Triacetin was obtained as a gift sample from Gattefosse India Pvt. Ltd., India. labrasol was procured from Loba Chemie, India. Trehalose was procured from HiMedia Laboratories Pvt. Ltd., India. Other chemicals and reagents used were of analytical grade.

Table 1: Solubility of NIMO in different oils, surfactants and co-surfactants and miscibility of selected oil with surfactant/co-surfactant.

Sl. No	Components	Materials	Solubility (mg/mL)	Miscibility of selected oil (Triacetin) with surfactant/co-surfactant
1	Oils	Triacetin	38.76	
		Peceol	2.908	
		Captex 200	18.039	
		Labrafac WL13349	12.84	
		Oleic acid	12.38	
		Isopropyl myristate	25.27	
2	Surfactants	Plurol disostearique	70.52	Miscible
		Labrafil M 1944CS	11.84	Turbid
		Cremophore RH 40	48.37	Clear
		Propylene glycol monolaurate	49.95	Clear
		Labrasol	54.29	Clear
		Tween 20	43.55	Turbid
		Span 80	49.88	Clear
		Span 20	44.21	Clear
		Triethanolamine	11.395	Clear
		Tween 80	38.947	Clear
3	Co-surfactants	Propylene glycol	32.25	Turbid
		PEG 600	43.55	Clear
		PEG 200	24.158	Turbid
		Ethanol	56.05	Clear
		Plurol oleque CC 497	64.29	Miscible

Methods

Screening and selection of components of nanoemulsion

Screening and selection of oil

An attempt had been made to screen different oils having different properties to predict the maximum solubility of NIMO in the oil. The solubility of NIMO in different oils was carried out by taking excess amounts of the drug in the fixed volume of different oils kept in each vial. The samples were maintained at 25± 0.5°C in the mechanical shaker and after 72 hr, they were subjected to centrifugation at 3000 rpm for 30 min using cooling centrifuge, Remi, India and followed by filtration.[6,18] The supernatant was withdrawn with the utmost care, and the concentration of the drug was estimated spectrophotometrically by means of a UV spectrophotometer at 351 nm.[19]

Screening and selection of surfactants and co-surfactants

The solubility of NIMO in different surfactants was carried out following the same procedure as that of screening of oils mentioned above. The miscibility study was performed with the selected oil in order to select a surfactant after screening and the results were presented in.[2,10] To predict the miscibility of surfactant in the selected oil, the mixing of surfactant and oil was made at the ratio of 1:1 employing a cyclo-mixer, Remi Lab world, India followed by visual observation. The co-surfactant was selected in the same way as that of the surfactant using miscibility studies with the selected oil. The visually clear appeared mixture was given due consideration for the fabrication of nanoemulsion. The solubility of Nimo in oil, surfactant and cosurfactant was portrayed in Table 1.

Construction of pseudo-ternary phase diagram to select the suitable region of nanoemulsion

The water titration method had been adopted to draw the pseudo ternary phase diagram of NIMO nanoemulsion in order to achieve a percentage range of nanoemulsion components for the existing range of nanoemulsion.[18,20] For the construction of each pseudo ternary phase diagram, the weight ratios of surfactant and co-surfactant were varied as 1:1, 1:2, 2:1, 3:2, and 2:3 respectively. Each pseudo ternary phase diagram was drawn with the weight ratios of oil to the S_{mix} at 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 respectively by using ProSim Ternary software.[21,22] Each oil: S_{mix} ratio was subjected to slow aqueous titration, and samples

Table 2: Independent variables and the experimental values of globule size (R1), PDI (R2), Percentage drug release in 15 min (Q15) based on D-Optimal Mixture Design with low and high limits.

Runs	Independent variables			Dependent variables		
	A (% Oil)	B (% S_{mix})	(% Water)	R1 (nm)	R2 (%)	R3 (%)
1	15	40	45	579	0.45	70
2	20	35	45	322.5	0.62	85
3	20	40	40	322.1	0.61	86
4	15	40	45	370.15	0.48	76
5	15	35	50	375.25	0.5	69
6	17.5	42.5	40	350.25	0.682	64
7	17.5	36.875	45.625	350.27	0.632	72
8	20	35	45	322.22	0.63	78
9	15	35	50	375.22	0.45	62
10	15	45	40	385.22	0.46	61
11	17.5	35	47.5	355.06	0.622	71
12	18.75	39.375	41.875	335.5	0.58	74
13	16.25	36.875	46.875	360.22	0.525	65
14	20	40	40	322.25	0.6	87
15	16.25	41.875	41.875	361.03	0.522	69
16	15	45	40	422.35	0.47	63
			Factors		Limits	
					Low (%)	High (%)
			Oill		15	20
			S_{mix}		35	45
			Water		40	50

were kept under visual observation for phase separation.[23,24] A transparent and easily flowable mixture was dotted in the phase diagram as o/w nanoemulsion.

Fabrication of NIMO nanoemulsion

At first, the oil phase was fabricated by the addition of the drug to the oil in a vial and vortexed for 5 min in a cyclo-mixer followed by homogenization and sonication for 10 min with 60% amplitude. The S_{mix} was prepared by mixing surfactant and co-surfactant at a 3:2 ratio as selected from the pseudo ternary phase diagram with the maximum area covered for the region of nanoemulsion followed by the addition of distilled water dropwise and again vortexed for 10 min.[16,23] The mixture of S_{mix} and water was added to the oil phase and vortexed for another 10 min to get transparent o/w nanoemulsion.

Formulation optimization of NIMO-nanoemulsion using optimal mixture design

From the literature study followed by preliminary experiment, the important CMAs and CPPs were identified and subjected to three components, two levels of optimal mixture design were selected as mentioned in Table 2, including 5 lack-of-fit points and five replicate points using Design-Expert 13 Stat-Ease for generating 16 runs.[24-26] The selected low and high ranges for oil, S_{mix}, and water in this investigation are fixed at 15-20%, 35-45%, and 40-50%, respectively, and the potential impact of independent variables such as % of oil, S_{mix}, and water on dependent variables such as Globule Size (GS), PDI and Q15 were evaluated, analyzed, and optimized both numerically and graphically. The formulations were subjected to lyophilization using trehalose as cryoprotectant.

Characterization of NIMO Nanoemulsion

Globule size, size Distribution, and zeta Potential

The average globule size (Z-Average) and size distribution of nanoemulsion was measured by employing Photon Correlation Spectroscopy (PCS). The principle involved in this technique is the measurement of variations of intensity of scattered light due to the Brownian movement of globules and allows measuring globule size by using the Stroke-Einstein equation. Before the measurement, the samples were suitably diluted. All the measurements were made by using a He- Ne laser at 633 nm, at 25°C, and at a fixed scattering angle of 90°. The surface charge of globules was measured by employing Zetasizer.[24] All the measurements were made after suitable dilution of the sample.

Percentage Transmittance

The percentage Transmittance of fabricated NIMO nanoemulsion was measured by employing a UV spectrophotometer. 1 mL of fabricated nanoemulsion was taken and diluted to 100 mL and analyzed under a UV spectrophotometer at 630 nm.[27]

Dispersibility Study

The efficacy of oral NIMO nanoemulsion was determined by performing a dispersibility test. Standard USP Dissolution apparatus II was used to study the dispersibility of fabricated nanoemulsion. Briefly, 2 mL of nanoemulsion was added to 500 mL of 0.1N HCl, and the experiment was conducted at 50 rpm and 37±0.5°C, and the Formulation was kept under visual inspection for the appearance of precipitation if any.[20,28]

Physical stability study

The physical stability of fabricated nanoemulsions was checked by heating and cooling cycles, centrifugation, and Freeze-Thaw cycle stress tests.[6,29,30] Briefly, the required volume of optimized nanoemulsion and required quantity of optimized lyophilized nanoemulsion were subjected to heating at 45°C in the hot air oven for 24 hr followed by cooling at room temperature (25±2°C) for the next 24 hr. The heating and cooling cycle was repeated six times, followed by centrifugation at 5000 rpm for 0.5 hr. Then it was kept at 2-8°C in the freezer for 24 hr followed by keeping it at room temperature for the next 24 hr, and the cycle was repeated six times. Then the GS, PDI, and ZP were determined and compared with the normally optimized nanoemulsion.[31-33]

Differential Scanning Calorimetry (DSC) studies

In order to investigate the physical state of the drug in the fabricated nanoemulsion and to predict possible potential interactions among various components with pure drugs, DSC was performed.[34] Briefly, weighed (Approximately 5 mg) sample was hermetically sealed and scanned between specific ranges of temperature with a heating rate of 10°C/min under constant nitrogen flow and compared with an empty aluminum pan as reference.

Fourier Transform Infra-Red (FTIR) studies

FTIR was performed to investigate for any chemical interactions between the drug and other components of nanoemulsion.[2,4,19,35] Before the FTIR study, the samples of pure drug and the lyophilized nanoemulsion were prepared by using the KBr pellets

Table 3: Observation and grading of nanoemulsion based on dispersibility test.

Grades	Dispersibility Test
A	Transparent and clear appearance of nanoemulsion within 1 min.
B	Appearance of slightly less clear nanoemulsion.
C	Milky appearance of emulsion within 2 min.
D	Dull white appearance indicating poor emulsification.
E	Poor emulsification due to appearance of oil droplets.

method, followed by recording spectra under a specific range of wave numbers.

Scanning Electron Microscopy (SEM)

The morphology and size of Globules were investigated by employing SEM.[19,20,28] The investigation was performed by keeping the optimized lyophilized NIMO nanoemulsion on a metal plate and dried under a vacuum to form a dry film, followed by observation under SEM.

Powder X-RD study

Patterns of X-ray powder diffraction of pure drug and lyophilized optimized Formulation were obtained by using an X-ray diffractometer with copper radiation CuKα (λ = 1.542 Å, 45 kV/40 mA), 2-theta range 2-40°, with step size 0.02° 2θ and time per step 300 sec.[12,28,36]

Entrapment Efficiency (EE): Entrapment Efficiency of fabricated NIMO nanoemulsion was determined by taking 5 mL of nanoemulsion in a 5 mL centrifuge tube and placed in a cooling centrifuge, and subjected to centrifugation at 20.000 rpm for 0.5 hr. The supernatant was collected and analyzed by using a UV spectrophotometer at 351nm, and the EE efficiency was calculated using the following formula

$$\%EE = \frac{\text{Total amount of drug taken} - \text{Free drug}}{\text{Total amount of drug taken}} \times 100$$

In vitro drug release study: The *in vitro* drug release study was performed by using the dialysis bag method.[14,19,21] The dialysis

Table 4: ANOVA for the quadratic model for the response of Globule size (GS), PDI and Q15.

ANOVA for the quadratic model for the response of Globule Size (GS)						
Source	**Sum of squares**	**d_f**	**Mean squares**	***F*-value**	***p*-value**	**Remarks**
Model	3.336E+05	5	66715.93	3.94	0.0310	Significant
(1)Linear Mixture	86901.92	2	43450.96	2.57	0.1259	
AB	37627.62	1	37627.62	2.22	0.1668	
AC	63281.11	1	63281.11	3.74	0.0819	
BC	1.860E+05	1	1.860E+05	10.99	0.0078	
Residual	1.692E+05	10	16923.57			
Lack of Fit	57118.93	5	11423.79	0.5095	0.7615	Not significant
Pure Error	1.121E+05	5	22423.35			
Cor Total	5.028E+05	15				
ANOVA for the linear model for the response of Polydispersity Index (PDI)						
Source	**Sum of squares**	**d_f**	**Mean squares**	***F*-value**	***p*-value**	**Remarks**
Model	0.2568	2	0.1284	4.59	0.0311	Significant
Linear Mixture	0.2568	2	0.1284	4.59	0.0311	
Residual	0.3640	13	0.0280			
Lack of Fit	0.1131	8	0.0141	0.2819	0.9451	Not significant
Pure Error	0.2508	5	0.0502			
Cor Total	0.6207	15				
ANOVA for quadratic model for the response of Q15						
Source	**Sum of Squares**	**d_f**	**Mean square**	***F*-value**	***p*-value**	**Remark**
Model	430.27	5	86.05	4.03	0.0291	Significant
Linear Mixture	225.60	2	112.80	5.28	0.0273	
AB	198.10	1	198.10	9.27	0.0124	
AC	177.44	1	177.44	8.30	0.0163	
BC	1.19	1	1.19	0.0557	0.8182	
Residual	213.73	10	21.37			
Lack of Fit	118.73	5	23.75	1.25	0.4063	Not significant
Pure Error	95.00	5	19.00			
Cor Total	644.00	15				

Table 5: Physical stability data of lyophilized nanoemulsion with different conditions.

parameters	Time in months						
	0	1		2		3	
Stability conditions		RT	Cold Condition	RT	Cold Condition	RT	Cold Condition
GS (nm)	322.5	322.85	323.25	323.65	324.22	323.75	324.23
PDI	0.451	0.473	0.478	0.482	0.489	0.501	0.512
EE	73	71.24	70.05	69.06	69.88	68.95	68.77
Visual appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear

RT-Room Temperature 25°C and Cold Condition 2-8°C.

Table 6: Important pharmacokinetic parameters obtained by oral administration of NIMO pure drug, NIMO-loaded nanoemulsion and lyophilized NIMO-loaded nanoemulsion.

Pharmacokinetic parameters	NIMO pure drug	NIMO-loaded nanoemulsion	Lyophilized NIMO-loaded nanoemulsion
C_{max} (µg/mL)	0.153±0.03	0.395±0.01	0.397±0.02
T_{max} (h)	1±0.05	0.5±0.04	0.5±0.02
AUC_{0-24} (µgh/mL)	7.005±1.5	10.797±1.2	15.587±2.8
t ½(h)	1.5±0.05	2.05±0.52	2.15±0.55
$AUC_{0-\infty}$ (µgh/mL)	8.035±1.5	21.65±2.5	23.89±2.6

NB- Mean ±standard deviation, *n*=6.

bag was kept in simulated gastric fluid overnight just before the day of the experiment. 5 mL of the fabricated nanoemulsion was kept inside the dialysis membrane, and both the ends were sealed and kept on the dissolution flask containing 900 mL of simulated gastric fluid in dissolution apparatus type II. The release study was conducted by maintaining the temperature of the release medium at 37±0.5°C and setting the speed at 50 rpm. At regular intervals of time, 5 mL of the sample was withdrawn, and the same volume was replaced with freshly prepared simulated gastric fluid. The release study was continued for 120 min. (20 min, 40 min, 60 min, 80 min, 100 min and 120 min followed by conduction of release study in simulated intestinal fluid in the same manner as in the simulated gastric fluid for another 2 hr.

In vivo pharmacokinetic study

In vivo pharmacokinetic study was performed using male albino rabbits with 2 kg each. All the rabbits were purchased by RIPS from Saha enterprises, 386/2 Nilachal, Birati, Kolkata-700051, India, with registration no-1828/PO/Bt/S/15/C PCSEA. The design protocol of the experiment was approved by Institutional Animal Ethical Committee (IAEC), RIPS, with protocol approval no IAEC RIPS/96/2019.

Grouping and selection of animals

Three samples vis-à-vis NIMO oral suspension optimized NIMO nanoemulsion, and lyophilized optimized NIMO nanoemulsion was given orally to albino male rabbits weighing 1.5 kg. Latin square crossover design was adopted for comparing the pharmacokinetic parameters of three samples. Six male albino rabbits were taken as subjects and three study periods were performed with a washout period of 2 days. The cross-over design involves no single rabbit can receive the same sample twice with a washout period of two days. The food and water which were provided to the selected rabbits twice daily should be hygienic and fresh. The calculation of the dose for every rabbit was made as follows.

Dose calculation

$$\text{Dose of albino rabbit} = \text{Total human adult dose} \times 0.07$$
$$(\text{Factor for each 1.5 kg weight of rabbit}) \times \text{weight of rabbit}/1.5$$

$$= (30\times0.07\times2)/1.5 = 2.8 \text{ mg}$$

Albino rabbits were administered orally with the help of Ryle's tube. Every time 0.5 mL of blood was withdrawn from the marginal ear vein of the rabbit by using needle no 24 and kept in Eppendorf tubes at sampling points of 0, 0.5 hr, 1 hr, 2hr, 4hr, 6hr, and 8 hr respectively after the required dose of administration. For serum collection, the blood was left uninterrupted in a slanting position for 0.5 hr to allow coagulation followed by centrifugation at 25°C at 3000 rpm for 10 min. The supernatant layer was separated using a micropipette. A solvent extraction technique was adopted for the extraction of NIMO from the serum sample. All-important pharmacokinetic parameters were calculated for all samples.

RESULTS

Screening and selection of oil

In this investigation, the solubility of NIMO in different oils such as Triacetin, Peceol, Captex 200, Labrafac lipophile WL 13349, Oleic acid, and Isopropyl myristate were tried, and observed the highest solubility in the Triacetin.

Screening and selection of surfactant and co-surfactant

In the current investigation, the solubility of the drug in different surfactants and co-surfactants was determined, and the highest solubility was observed in labrasol and Plurol Oleique CC 497 and hence selected as surfactant and co-surfactant respectively.

Construction of pseudo-ternary phase diagram to select the suitable region of nanoemulsion

A pseudo-ternary phase diagram was constructed to predict the best ratio of components of NIMO nanoemulsion (oil, surfactant, and co-surfactant).[17,35,37] The yellow solid area indicated the nanoemulsion region. A wider yellow region with more elongation towards the water(c) apex was observed in Figure 1(d), as portrayed in Figure 1 with a surfactant and co-surfactant ratio of 3:2. Therefore, S_{mix} ratio 3:2 was selected for the fabrication of nanoemulsion in this investigation.

Fabrication of NIMO nanoemulsion

A stable NIMO-nanoemulsion was fabricated successfully by using Design Expert-13, and the D-optimal mixture design was selected due to simple and the most suitable design for the fabrication of nanoemulsion using Triacetin as oil, labrasol as the surfactant, and Plurol Oleique CC497 as co-surfactant.

Formulation optimization of NIMO-nanoemulsion using D-optimal mixture design

As per the optimal mixture design, a total of 16 formulations were fabricated and subjected to evaluation for GS, EE, and PDI, and the values obtained from each formulation were put in the mixture design followed by analysis.[10,37] During analysis, predicted vs actual plots were obtained, which were portrayed in Figure 2. From the ANOVA analysis for GS, the predicted R^2 and Adjusted R^2 were found to be 0.9833 and 0.9981, respectively. As the difference between these values is within 0.02, the fit statistics are satisfied, but for Q15, fit analysis is not satisfied, and for PDI, the difference between the predicted R^2 and Adjusted R^2 is more than 0.02. The contour plots and 3D surface plots for GS, PDI, and Q15 were presented in Figures 3 and 4, and overlay plots in Figure 5 for predicted design space.

Characterization of NIMO nanoemulsion

FTIR and DSC

The FTIR spectra and DSC thermal spectra of the pure drug of NIMO and the lyophilized NIMO nanoemulsion were portrayed in Figures 6 and 7 respectively. The FTIR spectra of NIMO and lyophilized NIMO-loaded nanoemulsion revealed that there was neither additional nor missing nor significant shifting of peaks between the spectra of NIMO and nanoemulsion. The DSC peak revealed that the sharp endothermic peak was observed for NIMO obtained at 124.54°C which was close to the melting point of NIMO but in the case of lyophilized NIMO-loaded nanoemulsion, the peak was observed at 151.65°C.

X-RD, Globule size, Size Distribution, Zetapotential and SEM

The X-RD spectra were portrayed in Figure 8 which revealed that the crystallized structure of NOMO was retained even in the lyophilized product of NIMO-loaded nanoemulsion. The GS, PDI, and ZP of the optimized lyophilized nanoemulsion were found to be 322.1 nm, 0.453, and 13.5 mV, respectively, which were portrayed in Figure 9, and the SEM was presented in Figure 10.

Percentage transmittance, dispersibility study, and physical stability study

The obtained % transmittance and dispersibility values were portrayed in Table 3 and the physical stability was portrayed in Table 5. The % transmittance of the optimized formulation was found to be 99.58% which was closer to 100% indicating the optimized nanoemulsion was clear and transparent from the dispensability study the optimized formulation was found to be the transparent and clear appearance of nanoemulsion within 1 min and hence put under grade A. Physical stability data of lyophilized nanoemulsion with different conditions indicated that there was no any significant change of investigated parameters

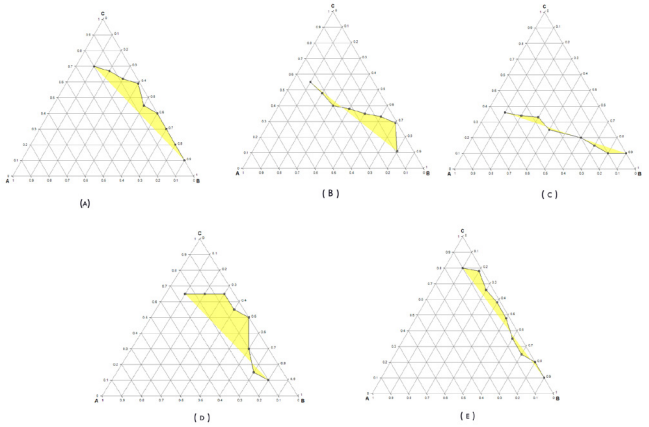

Figure 1: Ternary phase diagrams for different weight ratios (1:1, 1:2, 2:1, 3:2, and 2:3) of surfactant and co-surfactant.

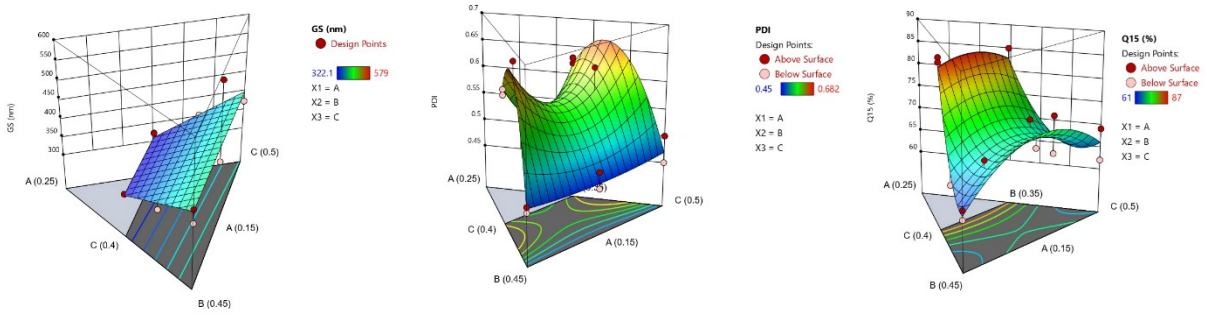

Figure 2: Predicted versus actual plots for globule size, PDI and Q15.

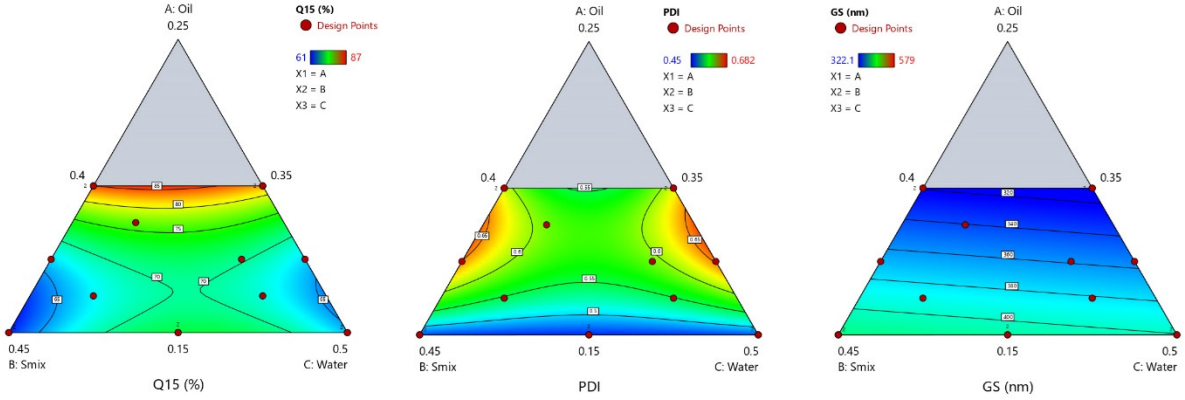

Figure 3: Contour plots for globule size, PDI and Q15.

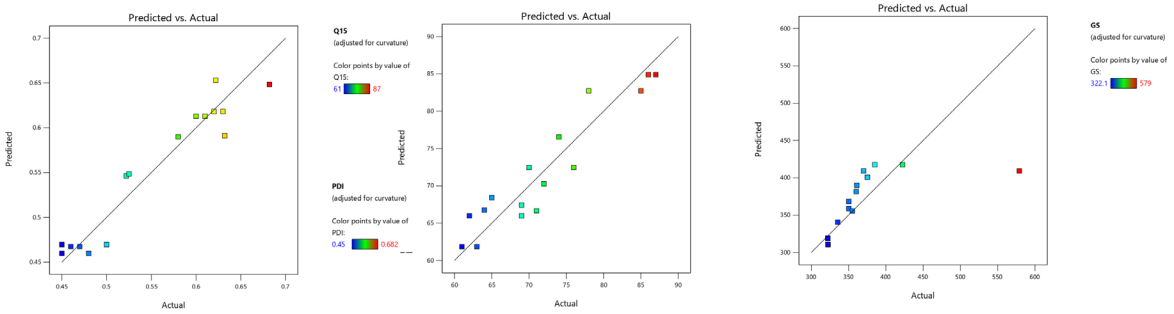

Figure 4: Surface plots (3D) for globule size, PDI and Q15.

hence the optimized nanoemulsion was found to be stable in lyophilized form.

In vitro drug release

The results of the *in vitro* drug release profiles of NIMO, optimized nanoemulsion, and optimized lyophilized nanoemulsion in simulated gastric fluid followed by intestinal fluid revealed that only 56%, 95%, and 91% of drug released from the pure drug of NIMO, optimized nanoemulsion and lyophilized nanoemulsion within 120 min. The maximum amount of drug released within 2 hr in simulated gastric fluid and the very negligible amount released in simulated intestinal fluid in the next 2 hr hence these data were not presented in the dissolution profile in graphical form. There was no significant change in drug release from the optimized nanoemulsion and lyophilized nanoemulsion. The *in vitro* dissolution graph was presented in Figure 11.

In vivo pharmacokinetic studies

The drug serum concentration vs. time plot was presented in Figure 12, and the important pharmacokinetic parameters i.e., C_{max}, T_{max}, and AUC were presented in Table 6. From the drug serum concentration versus time profile, it was observed that

the T_{max} and C_{max} of optimized nanoemulsion and optimized lyophilized nanoemulsion were more as compared to the pure drug of NIMO. The bioavailability of optimized nanoemulsion and optimized lyophilized nanoemulsion were also found to be significantly more as compared to NIMO.

DISCUSSION

The therapeutic oral use of NIMO is limited due to its short half-life, poor aqueous solubility, poor bioavailability, the requirement of more frequent administration of a drug, severe side effects, poor patient compliance, and inconvenience. In order to overcome these problems, NIMO nanoemulsion was developed by using the QbD approach with a built-in quality product.[1,19] From the preliminary screening design and preliminary experiment, the cause-and-effect relationship between CMAs and CPPs with CQAs was established, and appropriate CMAs and CPPs having a potential impact on CQAs were identified. In the present investigation, % of oil, % of S_{mix}, and % of water was identified as CMAs as they have a direct impact on CQAs (GS, PDI, and EE). Out of the different designs available on Design-Expert software, the D-Optimal mixture design was chosen as it is best suited for the selection of components and to optimize their quantities for the optimum Formulation of nanoemulsion[14,22,37] On analysis of the CQAs under consideration, GS was found to decrease with the increase in % of oil, decrease with the increase in % of S_{mix} and increase with the increase in % of water which is supported by the generated coded form of equation as,

$$GS=-808.75A - 340.92B + 365.15C + 1874.76AB + 2522.95AC + 1421.75BC$$

and the Q15 was also found to increase with the increase in % of oil, increase with % increase in S_{mix} and increase with % increase in water which can be presented in the form of coded equation as,

$$Q15=+152.71A + 66.05B + 60.64C - 136.03AB - 133.60AC - 3.06BC$$

and similarly, PDI was also found to decrease with a % increase in oil, increase with a % increase in S_{mix}, and increase with

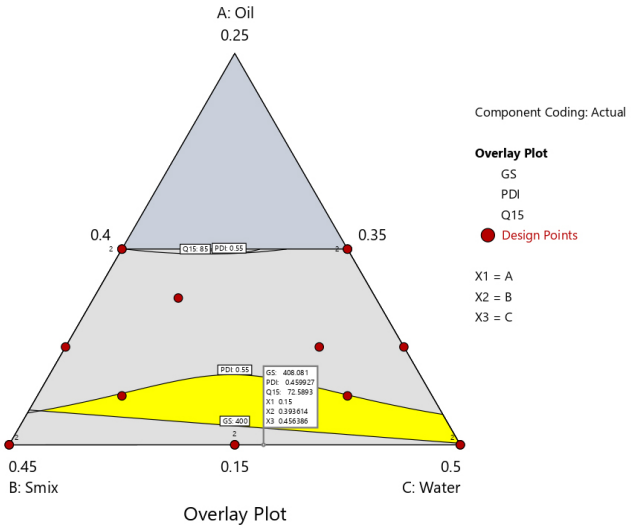

Figure 5: Overlays plots for GS, PDI, and Q15 to predict design space.

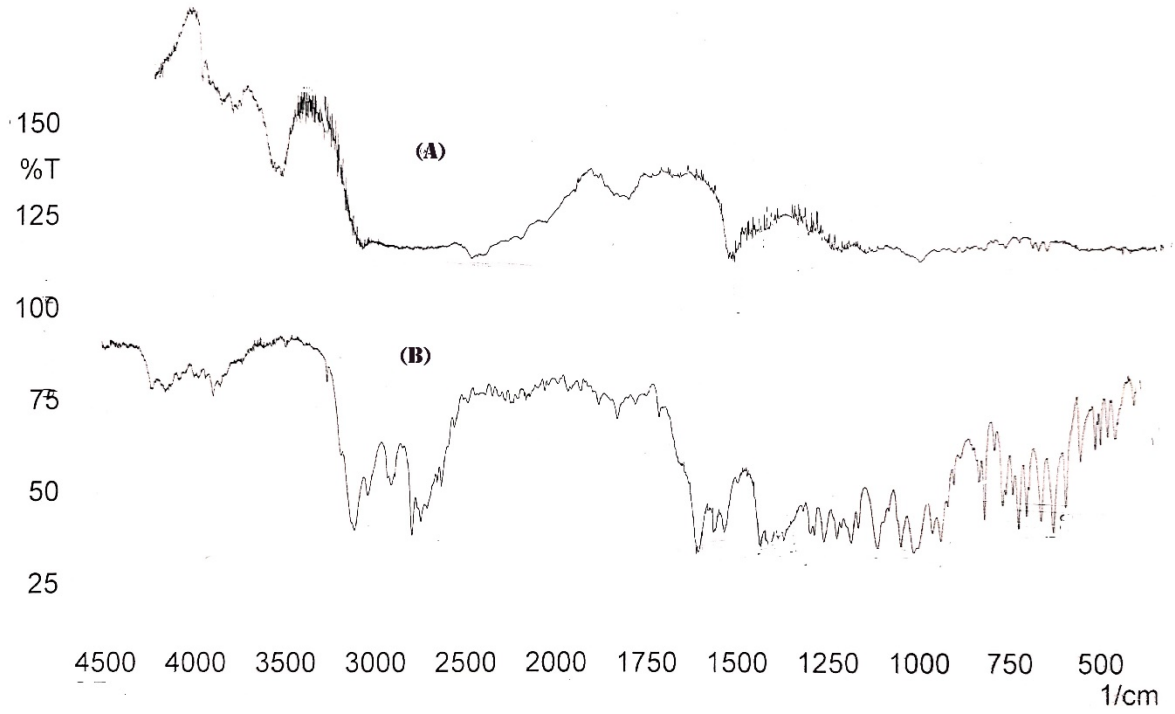

Figure 6: FTIR spectra of pure drug and optimized lyophilized formulation.

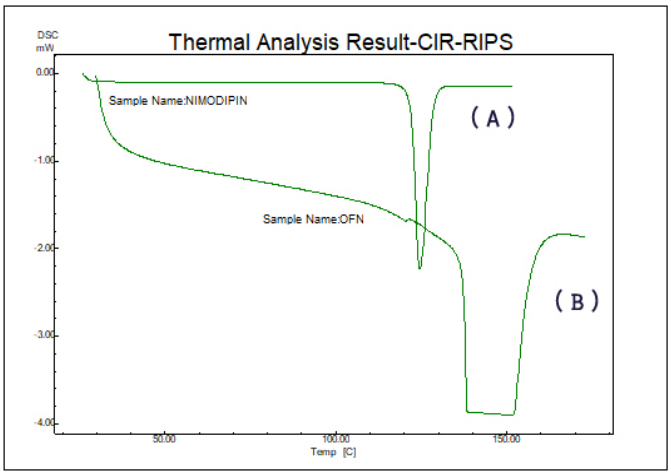

Figure 7: Thermal spectra (DSC) of pure drug and optimized lyophilized formulation.

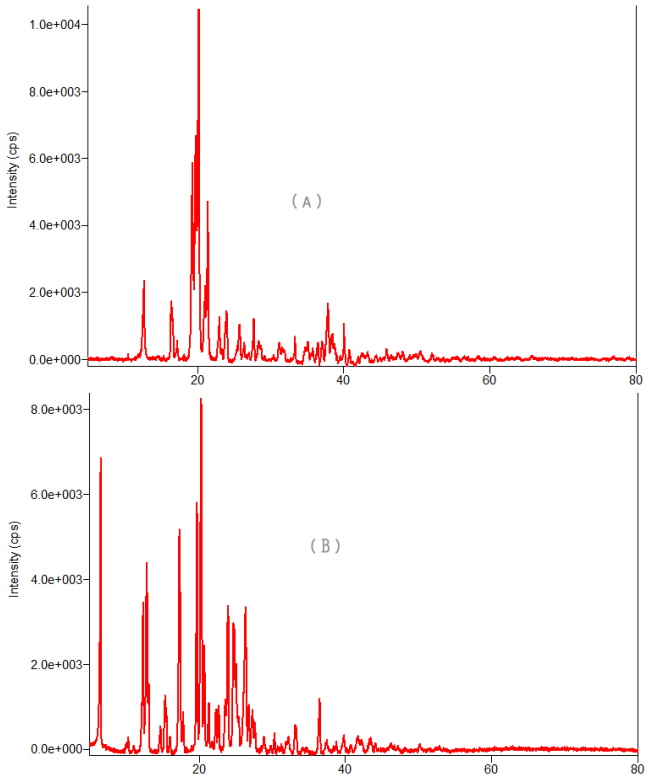

Figure 8: X-RD spectra of pure drug and optimized lyophilized formulation.

a % increase in water from the corresponding counterplots and 3D surface plots, which was also supported by the Design expert-generated coded equation as,

$$PDI = -5.30958A + 1.78038B + 1.6297C$$

From the graphical optimization, an overlay plot was obtained, which predicted the appropriate design space for optimum formulation and the ANOVA tables for significant of quadratic model of GS, PDI, and Q15 were presented in Table 4. The formulations were subjected to characterization for GS, Q15, PDI, ZP, Dispensability, % Transmittance, FTIR, DSC, X-RD,

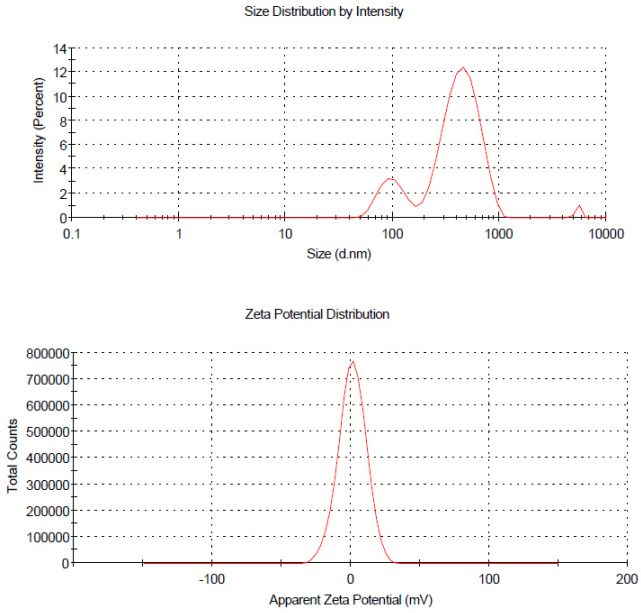

Figure 9: Average globule size, PDI, and Zeta potential of optimized formulation.

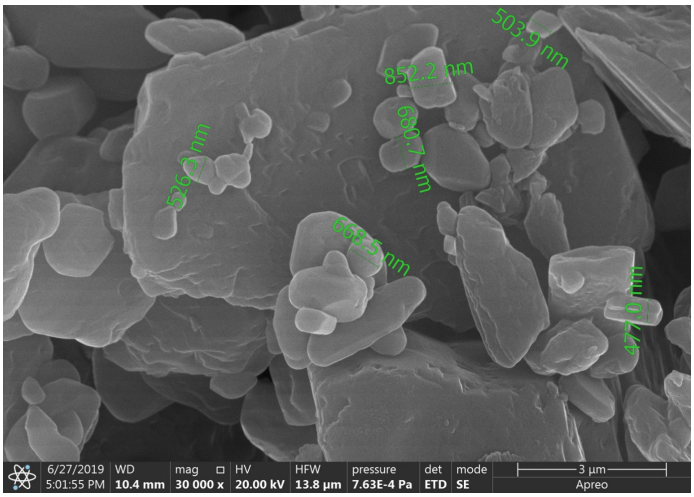

Figure 10: Scanning electron microscopy (SEM) of optimized formulation.

SEM, *in vitro* drug release by dialysis bag method and *in vivo* pharmacokinetic studies using rabbit model with Latin Square Design. The dispensability of data was presented in Table 3, which indicated that there was no precipitation in any formulation. It was observed that the value of % transmittance in the case of the optimized Formulation was closer to 100%, which indicated a clear and transparent appearance as previously reported by S. Kotta *et al.*[23] FT-IR was conducted been to predict the molecular interactions between NIMO and the lyophilized nanoemulsion containing NIMO. In the FT-IR spectrum as portrayed in Figure 6, the characteristic bands of NIMO at 3,296.9 cm-1, 1,309.5 cm^{-1} indicated the stretching vibration peak of N-H. The bands at 3,097.3 cm-1, 1,622.6 cm^{-1}, 1,494.9 cm^{-1}, and 808.8 cm^{-1} indicated a benzene ring. The ester group bands were at 1,685.3

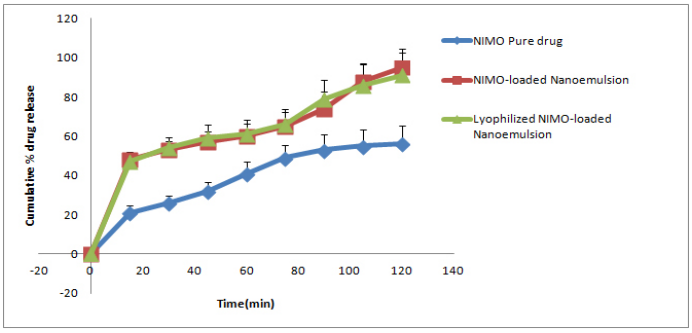

Figure 11: *In vitro* release profiles for pure drug, optimized nanoemulsion and lyophilized optimized nanoemulsion.

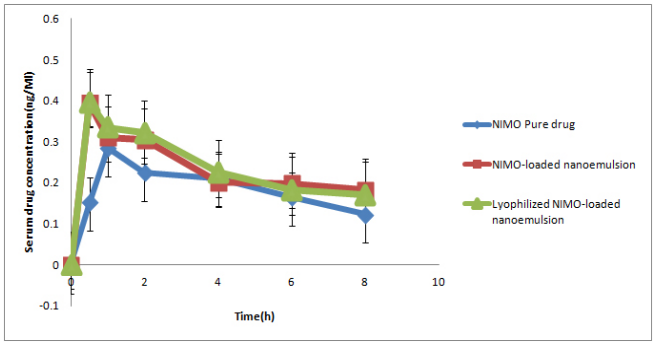

Figure 12: Serum drug concentrations versus time plots of pure drug, optimized nanoemulsion, and lyophilized optimized nanoemulsion.

cm-1, 1,280.6 cm^{-1}, and 1,032.6 cm^{-1}, -NO_2 was at 1,533.3 cm^{-1} and 1,356.2 cm^{-1}, C-O-C was at 1,099.8 cm^{-1}, C-H was at 2,982.2 cm^{-1}, 2,933.7 cm^{-1}, 1,454.8 cm^{-1}, and 1,381.6 cm^{-1} For the FT-IR data of NIMO-loaded-nanoemulsion, the new characteristic peaks of 3,452.4 cm^{-1}, 3,308.7 cm^{-1}, 2,915.1 cm^{-1}, 2,848.9 cm^{-1}, 1,747.9 cm^{-1}, and 1,538.5 cm^{-1} were observed. However, the intensity of the characteristic bands of NIMO were observably decreased in the nanoemulsion loaded with NIMO spectrum, and some peaks even disappeared, which suggested that there were no chemical interactions between the fNIMO and excipients used in nanoemulsion. All the results in this experiment were consistent with a previous reported study.[38]

The DSC peak revealed that the sharp endothermic peak was observed for NIMO obtained at 124.54°C which was close to the melting point of NIMO but in the case of lyophilized NIMO-loaded nanoemulsion, the peak was observed at 151.65°C. The thermal peaks corresponding to the DSC spectra of the drug and NIMO lyophilized nanoemulsion were found to be slightly different, which again confirmed there was no interaction between the drug and components of nanoemulsions.[39] The results were consistent with the results of in vitro release experiments in which the drug achieved a reasonable release rate due to crystalline state.

In the PXRD pattern of NIMO bulk drug, remarkable intense and distinct diffraction peaks of NIMO could be observed at two scattered angles of 6.546°, 12.468°, 11.982°, 17.287°, and 20.235° but the peaks patterns of the NIMO-loaded nanoemulsion were found to be at 11.255°, 12.324°. Hence the lyophilized NIMO-loaded nanoemulsion was observed to be less crystalline as compared to the pure drug of NIMO The X-RD spectra analysis of the drug and NIMO lyophilized nanoemulsion indicated that both the drug and lyophilized formulation were crystalline in nature. Scanning Electron Microscopy (SEM) indicated that different shapes of globules appeared in the optimized lyophilized Formulation. The in vitro drug release study indicated that the drug release from lyophilized nanoemulsion was better sustained as compared to the optimized nanoemulsion, which was due to the crystalline nature of the lyophilized formulation.

The analysis of physical stability data as presented in Table 5 indicated that the mean size of globules, PDI, and EE were 322.25 nm, 0.345 and 75% respectively. The results were found to be consistent throughout the investigation, which indicated that lyophilized Formulation was physically more stable as compared to pure drug and optimized nanoemulsion.

The serum drug concentration vs. time plot was presented in Table 6 which revealed that NIMO lyophilized formulation was found to have four times more bioavailability as compared to pure drug. The T_{max} was found to be more as compared to pure drugs. Yang D, *et al.*,[40] reported that oral nimodipine tablet has ½ α (h)= 2.30 ± 0.61, and Murali Mohan Babu GV *et al.*,[41] reported that oral nimodipine powder has t½ (h)= 1.64±0.037 similarly Kale AA *et al.*,[3] reported that oral nimodipine suspension has t½ (h)= 1.21±0.05 but in our investigation t½ (h) was found to be 1.5±0.05, 2.05±0.08 and 2.15±0.12 for pure drug, optimized NIMO-loaded nanoemulsion and lyophilized NIMO-loaded nanoemulsion respectively.

CONCLUSION

The NIMO nanoemulsion was fabricated successfully by implementing a QbD approach with the major components as Triacetin, labrasol, and Plurol oleique CC 497 as oil, surfactant, and co-surfactant, respectively. The most potential influencing CMAs on CQAs were identified and well established to produce in-built quality nanoemulsion fulfilling QTPP. The QbD-implemented lyophilized nanoemulsion was found to produce a three-fold increase in oral bioavailability and be more physically stable as compared to the pure drug of nimodipine.

ACKNOWLEDGEMENT

The authors are very much thankful to Roland Institute of pharmaceutical Sciences, Berhampur for providing required facilities for carrying out the research work of this manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

QbD: Quality by Design; **CQAs:** Critical Quality Attributes; **CMAs:** Critical Material Attributes; **CPPs:** Critical Process Parameters; **QTPP:** Quality Target Product Profile; **NIMO:** Nimodipine.

SUMMARY

Nimodipine is a BCS class II drug and it is also reported to have short half-life and poor oral bioavailability. To enhance oral bioavailability Nimodipine-loaded nanoemulsifying drug delivery system was developed. In this investigation, the three components triacetin as oil phase, labrasol as a surfactant, and plurol oleique CC 497 as co-surfactant were selected after screening. The ratio of surfactant and co-surfactant (S_{mix}) was selected from the pseudo-ternary phase diagram drawn by using ProSim ternary software. A d-optimal mixture design was employed to optimize the formulation. The Dynamic Light Scattering (DLS), FTIR, DSC, X-RD, SEM, *in vitro* drug release, stability study, and *in vivo* pharmacokinetic studies were carried out for the characterization of the optimized formulation and the results achieved the design objectives.

REFERENCES

1. Huang S, Huang Z, Fu Z, Shi Y, Dai Q, Tang S, *et al.* A novel drug delivery carrier comprised of nimodipine drug solution and a nanoemulsion: Preparation, characterization, *in vitro*, and *in vivo* studies. Int J Nanomedicine. 2020;(15):1161-72. doi: 10.2147/IJN.S226591.
2. Chircov C, Grumezescu AM. Nanoemulsion preparation, characterization, and application in the field of biomedicine [epub ahead of print]. 2019.
3. Kale AA, Patravale VB. Design and evaluation of Self-Emulsifying Drug Delivery Systems (SEDDS) of nimodipine. AAPS Pharm Sci Tech. 2008;9(1):191-6. doi: 10.1208/s12249-008-9037-9, PMID 18446481.
4. Beg S, Sandhu PS, Batra RS, Khurana RK, Singh B. QbD-based systematic development of novel optimized solid Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) of lovastatin with enhanced biopharmaceutical performance. Drug Deliv. 2015;22(6):765-84. doi: 10.3109/10717544.2014.900154, PMID 24673611.
5. Yukuyama MN, Kato ETM, de Araujo GLB, Löbenberg R, Monteiro LM, Lourenço FR, *et al.* Olive oil nanoemulsion preparation using high-pressure homogenization and D-phase emulsification – A design space approach. J Drug Deliv Sci Technol. 2019;49:622-31. doi: 10.1016/j.jddst.2018.12.029.
6. Ashaolu TJ. Nanoemulsions for health, food, and cosmetics: A review. Environ Chem Lett. 2021;19(4):3381-95. doi: 10.1007/s10311-021-01216-9, PMID 33746662.
7. Chávez-Zamudio R, Ochoa-Flores AA, Soto-Rodríguez I, Garcia-Varela R, García HS. Preparation, characterization and bioavailability by oral administration of O/W curcumin nanoemulsions stabilized with lysophosphatidylcholine. Food Funct. 2017;8(9):3346-54. doi: 10.1039/c7fo00933j, PMID 28856361.
8. Campolo O, Giunti G, Laigle M, Michel T, Palmeri V. Essential oil-based nanoemulsions: effect of different surfactants, sonication and plant species on physicochemical characteristics. Ind Crops Prod. 2020;157:112935.
9. Atsamnia D, Hamadache M, Hanini S, Benkortbi O, Oukrif D. Prediction of the antibacterial activity of garlic extract on *E. coli*, *S. aureus* and *B. subtilis* by determining the diameter of the inhibition zones using artificial neural networks. LWT Food Sciene Technol. 2017;82:287-95.
10. Khani S, Keyhanfar F, Amani A. Design and evaluation of oral nanoemulsion drug delivery system of mebudipine. Drug Deliv. 2016;23(6):2035-43. doi: 10.3109/10717544.2015.1088597, PMID 26406153.
11. Donsì F. Applications of nanoemulsions in foods. Nanoemulsions, formulation, application and characterization; 2018:349-77.
12. Silva HD, Cerqueira MÂ, Vicente AA. Nanoemulsions for food applications: development and characterization. Food Bioprocess Technol. 2012;5(3):854-67. doi: 10.1007/s11947-011-0683-7.
13. Inal A, Yenipazar H, Şahin-Yeşilçubuk N. Preparation and characterization of nanoemulsions of curcumin and echium oil. Heliyon. 2022;8(2):e08974. doi: 10.1016/j.heliyon.2022.e08974, PMID 35243093.
14. Acharya SD, Tamane PK, Khante SN, Pokharkar VB. QbD based optimization of curcumin nanoemulsion: DoE and cytotoxicity studies. Indian J Pharm Educ Res. 2020;54(2):329-36. doi: 10.5530/ijper.54.2.38.
15. Singh B, Beg S, Sharma G, Jain A, Negi P. Holistic Application of Quality by Design (qbd) for Pharma Product Development excellence and Regulatory Compliance. Nirma Univ J Pharm Sci. 2014;1:19-35.
16. Adena SKR, Herneisey M, Pierce E, Hartmeier PR, Adlakha S, Hosfeld MAI, *et al.* Quality by design methodology applied to process optimization and scale up of curcumin nanoemulsions produced by catastrophic phase inversion. Pharmaceutics. 2021;13(6):880. doi: 10.3390/pharmaceutics13060880, PMID 34203672.
17. Tripathi CB, Parashar P, Arya M, Singh M, Kanoujia J, Kaithwas G, *et al.* QbD-based development of α-linolenic acid potentiated nanoemulsion for targeted delivery of doxorubicin in DMBA-induced mammary gland carcinoma: *in vitro* and *in vivo* evaluation. Drug Deliv Transl Res. 2018;8(5):1313-34. doi: 10.1007/s13346-018-0525-5, PMID 29748834.
18. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech. 2015;5(2):123-7. doi: 10.1007/s13205-014-0214-0, PMID 28324579.
19. Chalikwar SS, Belgamwar VS, Talele VR, Surana SJ, Patil MU. Formulation and evaluation of nimodipine-loaded solid lipid nanoparticles delivered via lymphatic transport system. Colloids Surf B Biointerfaces. 2012;97:109-16. doi: 10.1016/j.colsurfb.2012.04.027, PMID 22609590.
20. Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK, *et al.* Nanoemulsion: concepts, development and applications in drug delivery. J Control Release. 2017;252:28-49. doi: 10.1016/j.jconrel.2017.03.008, PMID 28279798.
21. LIANG CX, QI DL, ZHANG LN, Lu P, Liu ZD. Preparation and evaluation of a water-in-oil nanoemulsion drug delivery system loaded with salidroside. Chin J Nat Med. 2021;19(3):231-40. doi: 10.1016/S1875-5364(21)60025-0, PMID 33781457.
22. Maher PG, Fenelon MA, Zhou Y, Kamrul Haque M, Roos YH. Optimization of β-casein stabilized nanoemulsions using experimental mixture design. J Food Sci. 2011;76(8):C1108-17. doi: 10.1111/j.1750-3841.2011.02343.x, PMID 22417574.
23. Kotta S, Khan AW, Ansari SH, Sharma RK, Ali J. Formulation of nanoemulsion: A comparison between phase inversion composition method and high-pressure homogenization method. Drug Deliv. 2015;22(4):455-66. doi: 10.3109/10717544.2013.866992, PMID 24329559.
24. Dordević SM, Radulović TS, Cekić ND, Ranđelović DV, Savić MM, Krajišnik DR, *et al.* Experimental design in formulation of diazepam nanoemulsions: physicochemical and pharmacokinetic performances. J Pharm Sci. 2013;102(11):4159-72. doi: 10.1002/jps.23734, PMID 24114833.
25. Ferreira-Nunes R, Gratieri T, Gelfuso GM, Cunha-Filho M. Mixture design applied in compatibility studies of catechin and lipid compounds. J Pharm Biomed Anal. 2018;149:612-7. doi: 10.1016/j.jpba.2017.11.069, PMID 29202440.
26. Nagi A, Iqbal B, Kumar S, Sharma S, Ali J, Baboota S. Quality by design based silymarin nanoemulsion for enhancement of oral bioavailability. J Drug Deliv Sci Technol. 2017;40:35-44.
27. Rao S, Barot T, Rajesh KS, Jha LL. Formulation, optimization and evaluation of microemulsion based gel of butenafine hydrochloride for topical delivery by using simplex lattice mixture design. J Pharm Investig. 2016;46(1):1-12. doi: 10.1007/s40005-015-0207-y.
28. Dasgupta N, Ranjan S. Food nanoemulsions: stability, benefits and applications. In: An introduction to food grade nanoemulsions. Environmental chemistry for a sustainable world. Singapore: Springer; 2018:19-48. doi: 10.1007/978-981-10-6986-4_2.
29. Che Marzuki NH, Wahab RA, Abdul Hamid M. An overview of nanoemulsion: concepts of development and cosmeceutical applications. Biotechnol Biotechnol Equip. 2019;33(1):779-97. doi: 10.1080/13102818.2019.1620124.
30. Majeed A, Bashir R, Farooq S, Maqbool M. Preparation, characterization and applications of nanoemulsions: an insight. J Drug Deliv Ther. 2019;9(2):520-7. doi: 10.22270/jddt.v9i2.2410.
31. Gao Y, Liu Q, Wang Z, Zhuansun X, Chen J, Zhang Z, *et al.* Cinnamaldehyde nanoemulsions; physical stability, antibacterial properties/mechanisms, and biosafety. J Food Meas Char. 2021;15(6):5326-36. doi: 10.1007/s11694-021-01110-6.
32. Feng J, Wang R, Chen Z, Zhang S, Yuan S, Cao S, *et al.* Formulation optimization of D-limonene-loaded nanoemulsions as a natural and efficient biopesticide. Colloids Surf A Physicochem Eng Aspects. 2020;596:124746.
33. Du Z, Wang C, Tai X, Wang G, Liu X. Optimization and characterization of biocompatible oil-in-water nanoemulsion for pesticide delivery. ACS Sustain Chem Eng. 2016;4(3):983-91.
34. Abbas S, Hayat K, Karangwa E, Bashari M, Zhang X. An overview of ultrasound-assisted food-grade nanoemulsions. Food Eng Rev. 2013;5(3):139-57. doi: 10.1007/s12393-013-9066-3.
35. Sharma P, Tailang M. Design, optimization, and evaluation of hydrogel of primaquine loaded nanoemulsion for malaria therapy. Future J Pharm Sci. 2020;6(1):26-7. doi: 10.1186/s43094-020-00035-z.
36. Borthakur P, Boruah PK, Sharma B, Das MR. Nanoemulsion: preparation and its application in food industry A2. Grumezescu, Alexandru Mihai BT – emulsions. Elsevier Inc 2016.
37. Samson S, Basri M, Fard Masoumi HR, Abedi Karjiban R, Abdul Malek E. Design and development of a nanoemulsion system containing copper peptide by D-optimal mixture design and evaluation of its physicochemical properties. RSC Adv. 2016;6(22):17845-56. doi: 10.1039/C5RA24379C.

38. Teng Z, Yu M, Ding Y, Zhang H, Shen Y, Jiang M, *et al*. Preparation and characterization of nimodipine-loaded nanostructured lipid systems for enhanced solubility and bioavailability. Int J Nanomedicine. 2019;14:119-33. doi: 10.2147/IJN.S186899, PMID 30613141.
39. Hsieh IT, Chang JS, Chou TH. The impact of the surfactant type on physicochemical properties, encapsulation, and *in vitro* biocompatibility of coconut oil nanoemulsions. J Taiwan Inst Chem Eng. 2022;137.
40. Yang D, Zhu J, Zheng Y, Ge L, Zhang G. Preparation, characterization, and pharmacokinetics of sterically stabilized nimodipine-containing liposomes. Drug Dev Ind Pharm. 2006;32(2):219-27. doi: 10.1080/03639040500466270, PMID 16537202.
41. Gedela V, Babu MM, Kumar NR, Kasina H, Sankar BJ, Namburu K, *et al*. *In vivo* evaluation of modified gum karaya as a carrier for improving the oral bioavailability of a poorly water-soluble drug, nimodipine. AAPS Pharm Sci Tech;3:1-9.

Cite this article: Das P, Panda J, Jena GK, Patra CN, Panigrahi KC. Design Expert-Implemented Nimodipine-Loaded Lyophilized Nanoemulsifying Drug Delivery System for Improved Oral Bioavailability and Physical Stability. Indian J of Pharmaceutical Education and Research. 2024;58(1s):s126-s138.