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Phase Preparation of Lyophilized Nanoemulsion of Nimodipine for Enhanced Stability and Biopharmaceutical Presentation

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Abstract: ***Purpose*:** The primary objective of the current research is to develop a novel lyophilized nanoemulsion (NM) of Nimodipine (ND) to improve its oral bioavailability and stability. ***Method*:** Triacetin, cremophor RH40 and PEG600 were identified as oil, surfactant, and co-surfactant respectively. It was then optimized using BBD and the optimized nanoemulsion was further lyophilized with trehalose as cryoprotectant to improve the long-term storage stability. ***Result*:** NM and its lyophilized NM showed globule size less than 200 nm with more than 75 % drug diffusion within 30 min. Pharmacokinetic study for lyophilized nanoemulsions exhibited fivefold increase in bioavailability. Lyophilized F4 showed improved stability due to conversion of nanoemulsion in to solid form by lyophilisation using trehalose as cryoprotectant. ***Conclusion*:** Hence lyophilized nanoemulsion of ND can be prepared for improved oral bioavailability and stability.

Keywords: Diffusion Study, Gobule size, Pharmacokinetic study, Thermodynamic stability
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1. INTRODUCTION

Hypertension is a prime risk factor for cardiovascular diseases, which may lead to critical health issues by increasing the risk of heart stroke/attack/heart failure and aneurysm. Nimodipine (ND) is a calcium channel blocker that can act as an anti-hypertensive [1]. It is also used in preventing a major complication of subarachnoid hemorrhage. However, the drawback of this particular drug is its poor oral bioavailability, i.e., 13 % due to significant first-pass metabolism [2]. ND is poorly water-soluble with a log P of 3.41 [3]. Currently, lipophilic drugs are incorporated into inert vehicles made of lipids which are utilized for the improvement of oral drug delivery. Lipids can play a significant role in the improved delivery of lipophilic drugs with low oral bioavailability by virtue of their higher solubilization capacity. Nanoemulsion (NE) is preferred among other approaches because of the following attributes *vis-a-vis* quick dispersion, low inter-subject variability, the biocompatibility of lipids, lymphatic absorption, and practicability of industrial-scale manufacturing [4]. Many studies were executed to enhance oral delivery of drugs like amlodipine [5], felodipine [6], and mebudipine [7] by nanoemulsion technique.

NEs are thermodynamically stable mixtures with globule sizes below 100 nm. Nanoemulsions are multiple (W/O/W) or biphasic nanoemulsions (W/O or O/W) as per the relative distribution and components of the internal dispersed phase/ phases and the very common continuous phase. The overall

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stability and droplet number of the nanoemulsion is governed by the phase volume ratio, which measures the relative volumes of the external and internal phase [8]. Lyophilization process has been attempted by many nanoemulsions for getting solid powder formulations for better stability [9]. The primary objective of the current research is to develop a novel lyophilized nanoemulsion of ND to improve its oral bioavailability and stability.

2. MATERIALS AND METHODS

Materials

ND was a gift sample from Dr. Reddy's Lab, Hyderabad, India. Cremophor and triacetin were obtained as a gratis sample from Gattefosse India Pvt Ltd, India. PEG600 was procured from Loba Chemie, India. Trehalose was procured from Himedia India Ltd, India. Other chemicals and reagents used were of analytical grade.

Methods

Solubility screening:

The solubility study of ND in different surfactants, co-surfactant, and oil was assessed. 1 mL oil/surfactant/co-surfactant was taken in a 5 mL vial, and an excess amount of ND was added, then it was mixed for 5 min using cyclomixer and shaken in a water bath shaker [10]. Subsequently, the suspension was centrifuged at 8000 rpm for 10 min. The supernatant was then collected and diluted with solvent and analyzed spectrophotometrically at 351 nm [11].

Construction of phase diagram

The Smix (surfactant: co-surfactant) were mixed at different ratios (1:1, 1:2. 2:1, 2:3 and 3:2) to fulfil the HLB value requirements. Different proportions of oil: Smix were prepared. Then titration was carried out slowly with the aqueous phase. Samples were observed visually for any phase separation. Phase diagrams were then constructed using PRO SIM Ternary phase diagram software 1.0 [12,13]. Easily flowable and transparent mixtures were scattered on the phase diagram as o/w NE.

Preparation of NE

The phase containing oil was prepared by adding ND and vortexed for 5 min. In the aqueous phase, Smix and distilled water were vortexed for 5 min. Then oil phase was gradually added to aqueous phase with high-speed stirring at 25,000 rpm using Ultraturrex, Ika, Germany) for 5 min. The formed coarse emulsion was then probe sonicated at 40% amplitude for 3 min with 4 S/2 S on and off-cycle, respectively (Sonics 500, USA). The process of probe sonication was performed by keeping the emulsion in an ice bath [14].

BBD enabled optimisation and analysis

Systematic optimization of nanoemulsion formulations of ND was accomplished employing box-behnken design (BBD) with the help of design expert ver. 11.1.01 software (Stat-Ease, Minneapolis, MN). The relative impact of Triacetin, S mix and water on different critical quality attributes (CQAs) like drug content (%), globule size (nm) and cumulative % drug release at 30 min (Q30%) were analysed in terms of regression equation and response surface. Optimisation were carried out to construct the overlay plot and to identify the design space.

Thermodynamic Stability

NEs were subjected to centrifugation (5000 rpm for 30 min) and observed for phase separation, creaming and cracking [15,16]. NEs were exposed to 6 cycles of 4ºC and 45ºC (48 h) and were observed for cracking/creaming/separation. 3 freeze-thaw cycles of 48 h were executed for the NEs between the temperatures -21 ºC and +25 ºC. Instability-like phase separation was inspected [17].

Lyophilization of NE

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Frozen NE was lyophilized at -52^{0}C using trehalose as a cryoprotectant (10, 20, and 30 % w/v). The Lyophilization process was continued for 48 h to obtain free-flowing powders [18,19].

Characterization of Formulations

Drug Content

An amount equivalent to 1 mg of ND was taken from NE and lyophilized NE in a volumetric flask. 1 mL of dimethylformamide (DMF) was added to it and vortexed for 2 min. Then it was filtered, diluted and absorbance was measured at 351 nm spectrophotometrically [20,21].

Dispersibility Test

A dispersibility test was performed in the dissolution apparatus. 1 mL formulation was mixed with 500 mL of distilled water (37±0.5ºc), and the dissolution paddle was rotated at 50 rpm. Then the formation of the emulsion was observed visually and categorized [22,23].

Particle size (PS), Zeta potential (ZP), and Polydispersity Index (PDI)

NE and lyophilized NE were diluted ten times with distilled water and measured for PS, ZP, and PDI by using zeta sizer (Malvern, nano-ZS-90, JK) [24,25].

***In vitro* Diffusion Study**

The *In-vitro* drug release study was performed for pure drug ND, NE, lyophilized NE, and marketed tablet using dialysis membrane. NE equivalent to 30 mg of ND was loaded into the dialysis bag in order to perform the diffusion study. The dialysis membrane bag is placed inside 0.1 N HCl for two h. Aliquot of 2 ml were collected at 15 min for two h and analyzed spectrophotometrically at 351 nm. The diffusion data were used for the determination of dissolution efficiency [DE_{30} (%)], mean dissolution time (MDT), and percent drug released at 30 min [Q_{30} min (%)] [26,27].

FT-IR Spectroscopy Study

FT-IR study was done to ascertain the compatibility between ND and excipients used in the formulation of lyophilized NE. This study was performed by using IR Affinity-1, (Shimadzu, Japan) [28,29].

Differential Scanning Calorimetry (DSC) Study

DSC thermogram of ND and lyophilized F4 was conducted at a heating rate 10^0 C/min from 25 to 250ºC in aluminum pans (DSC-60, Shimadzu, Japan)C [30,31].

Pharmacokinetic (PK) Study

Analytical Method

A reported method with the following specifications was used for the estimation of ND in rabbit serum, i.e., C18 column, mobile phase [acetonitrile: water(70:30)], flow rate 1mL/min, PDA detection (351 nm), and extracting solvent (diethyl ether) [32].

Grouping and selection of animals

Three samples, namely ND (aqueous suspension), optimized NE, and lyophilized NE, were administered orally to white albino rabbits by using a Latin square crossover design. In this study, six rabbits received samples at three different study periods, i.e., 1, 2, and 3. The dose administered to rabbits was 3 mg. 0.5 mL of blood was sampled (marginal ear vein) at several time points 0, 1, 4, 8, 12, 24 h. The animal experiments complied with the ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The current PK study was approved (approval no 103) by IAEC of Roland institute of pharmaceutical sciences (Regd. No. 926/PO/ac/06/CPCSEA). Serum samples were centrifuged, the supernatant was collected and extracted with diethyl ether, the dry extract was diluted with mobile phase, and finally, the ND content was estimated by UFLC, and the various PK parameters were determined [33,34].

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Stability Study

It was performed with reference to ICH Q1A (R20 guidelines at 25±2◦C/60±5% RH for three months (15). Optimized NE, and lyophilized NE were analyzed for different CQAs at 1 month interval for 3 months [35].

3. RESULTS AND DISCUSSION

Solubility Screening

Solubility study for ND exhibited maximum solubility in triacetin, cremophor RH40, and PEG600 as oil, surfactant, and co-surfactant, respectively (Figure 1). The solubility of ND in triacetin was found to be 39.5 ± 1.22 mg/mL, which in comparison to its water solubility (0.012 ± 0.001 mg/mL), it represents the potential of this oil to solubilize ND. ND also exhibited high solubility in cremophor RH 40 (50.4 ± 1.32) i.e. surfactant and PEG600 (44.3 ± 0.76) i.e. co-surfactant. Higher solubility of ND in surfactant and co-surfactant than oil can be attributed to the amphiphilic nature of surfactant molecules with affinity for both polar and nonpolar groups. Another pivotal component of NE was stability imparted by surfactant and flexibility to interfacial film by co-surfactant [36].

Phase Diagram

The optimum ratio of oil, surfactant, and co-surfactant was selected by phase solubility diagram using dotted lines. From the results, a wider NE region was obtained for the 1:1 ratio of Smix (Figure 2). Increasing the surfactant concentration further, i.e., 2:1 or co-surfactant concentration further, i.e., 1:2 resulted in a lower NE region. Similarly, lower NE regions for 2:3 and 3:2 were also observed.

Preparation of NE

Table 1 represents composition of NE formulations of ND using 3-factor at 3-level BBD. Seventeen formulations were optimised taking Triacetin as Oil, Cremophor RH 40 as surfactant, and PEG 600 as co-surfactant. The ration between Cremophor RH 40 and PEG 600 was fixed at 1:1 as per the result in phase diagram study. The concentration of surfactant and co-surfactant mixture was varied in different formulation and further evaluated for different study parameter.

BBD enabled optimisation and analysis

The regression equation represents the relative impact of Triacetin, S mix and water on various CQAs. Following are the polynomial equation obtained in terms of coded factor for each CQA.

$$Drug\ Content(\%) = 65.58 - 4.29A + 12.63B - 1.09C + 0.075AB - 1.8AC + 0.725BC + 1.43\ A^2 + 2.86B^2 - 1.02C^2$$

$$Globule\ Size = 341.4 + 56A - 198.13B + 14.88C + 26.75AB + 6.25AC + 16.50BC + 32.3\ A^2 - 21.95B^2 + 2.55C^2$$

$$Q30\% = 61.34 - 4.17A + 15.60B + 0.57C - 4.3AB + 3AC - 3BC$$

Figure 3 represents Contour plot and 3D plot showing the effect of Triacetin, S mix and water on various CQAs. By observing the plot, it can be predicted that there is a quadratic relationship of drug content (%) with that of the conc. of S mix and Triacetin. High % of drug content observed when there is increase in the conc. of Smix and decrease in the conc. of Triacetin. In case of globule size, it was observed that at high level of Smix favours the smaller globule size whereas high conc. of Triacetin and water promotes larger globule size. The Contour plot and 3D plot for Q30 indicating that Q30 (%) gradually increases with increment in the conc. of Smix. Whereas there is a reverse effect when there is an increment in the conc. of Triacetin.

Table 2 represents the summary of ANOVA for different factor and its significance with respect to quadratic model. The Model F-value of 48.73 implies the model is significant as per CQA drug content. P-values less than 0.0500 indicate model terms are significant. In this case A, B, B^2 are

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significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Model F-value of 164.84 implies the model is significant for CQA globule size. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, A^2, and B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Model F-value of 78.90 implies the model is significant for Q30%. P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, AC, and BC are the significant model terms.

For the purpose of obtimistion the desirable goal for different CQAs were fixed with appropriate weightage which were then processed for optimisation. The desirability for drug content(%) was fixed in the range of 70 to 100 %. In case of globule size (nm) the desirability was fixed in the range of 10 to 200 nm. Q30 (%) was fixed in the range of 70 to 100% for achieving the desired quality attributes. On the basis of the desirability for different CQAs the design space was identified as yellow region in the working space in the overlay plot as portrayed in Figure 4. It also depicts the selected optimised NE of ND composition which is same as F4 formulation in the experimental run. The optimised formulation consists of 200 mg of Triacetin as oil, 100 mg of Cremophor RH 40 as surfactant, 100 mg of PEG 600 as co-surfactant, and 150 mg of water.

Thermodynamic Stability (TS)

TS study is (centrifugation, heating–cooling cycles, and freeze-thaw cycles) is of major significance to annihilate metastable NEs and to choose a formulation with longer shelf life. Formulations F1, F2, F9, and F11 could not pass the TS test, whereas other formulation passed the TS test. This reveals that a higher amount of Smix, i.e., at least 150 mg, is needed to get a stable emulsion.

Lyophilization

The optimized NE (F4) was lyophilized with 10, and 20 % of trehalose w/v were not free-flowing, whereas 30 % trehalose exhibited desirable flowability. Lyophilized NE (F4) with 30 % w/v of trehalose was selected.

Drug content

The drug content was found to be greater than 85 % for NE (F4) and lyophilized NE (F4) (Table 3). The solubility of the drug in the oily phase is an important factor in designing a formulation because drug loading in formulation, the ability of the formulation to keep the drug in the solubilized form in GI tract, and volume of formulation for delivery of therapeutic dose directly depend greatly on its solubility in the vehicle [37].

Dispersibility test

The *In vitro* execution of the NE (F4) and lyophilized F4 were analyzed in the naked eye by a grading system (Table 3). NE formulation F4 passed the dispersibility test in Grade-A (speedily formed NE within 1 min, showing a lucid or bluish look). This can be attributed to the optimum proportion of surfactant and co-surfactant. The selection of Cremophor RH 40 and PEG 600 as surfactant and co-surfactant respectively contributed in producing a transparent emulsion. Lyophilized formulation (F4) also passed the dispersibility test in Grade A, suggesting faster dispersibility due to a tremendous increase in surface area and faster dissolution. When the NE interacts with GI fluids, desorption of the surfactant layer takes place at the globule interface to retain aqueous phase concentration corresponding to its CMC.

Globule size, Zeta potential, and Polydispersity Index (PDI)

Table 3 also shows the result of different study parameter like Globule size, Zeta potential, and Polydispersity Index (PDI). The globule size of NE formulation F4 was 76 nm which fulfils the requirement or specifications of NE. Higher zeta potential of – 26.5 mV suggests globules remain separate from each other preventing agglomeration and contributing to the formulation of a stable emulsion. Polydispersibility index (PDI) less than 0.3 indicates uniform size distribution. Lyophilized NE F4 exhibited an increase in globule size from 76 to 196 nm. This increase in globule size could be

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due to the fusion of particles. However, the zeta potential and PDI values for lyophilized NE F4 did not show any significant change ($P < 0.05$ level).

Diffusion Study

Figure 5 represents *In vitro* diffusion study pure drug ND, Nanoemulsion formulation (F4), Lyophilized nanoemulsion of F4, and marketed tablet of ND. Table 4 represents the values of diffusion efficiency at 30 min (DE_{30}), percent drug diffusion at 30 min (Q_{30}), and mean dissolution time (MDT) of pure drug ND, Nanoemulsion formulation (F4), Lyophilized nanoemulsion of F4, and marketed tablet of ND. The percent drug diffusion from the pure drug, NE F4, marketed tablet, and lyophilized NE (F4) at 30 min (Q_{30}) was found to be 3.5, 83.5, 17.3, and 79.3 %, respectively. The Q_{30} values showed a 24-fold and 23-fold increase in diffusion rate at 30 min for NE formulation F4 and lyophilized NE (F4), respectively, in comparison to pure drug. These formulations also showed nearly five times improvement in Q_{30} values in comparison to the marketed tablet. Similarly, diffusion efficiency (DE_{30}) values showed a significant increase in diffusion efficiency of formulations. The *MDT* for pure drug ND and marketed tablet is 63.12 and 56.85 min, respectively. It decreased to 18.81 and 21.33 min for NE (F4) and lyophilized formulation (F4), respectively. Droplets in the nanosize range with a very large surface area were responsible for a higher diffusion rate for NE F4 and lyophilized F4 in collating to pure drug ND and marketed tablet.

FT-IR Spectroscopy Study

The FT-IR spectra (Figure 6) showed the presence of the following functional groups such as NH, ester, NO_2, and C-O-C at 3295, 1695, 1520, and 1099 cm^{-1}, respectively. The lyophilized formulations also showed the presence of similar functional groups. Hence, the drug and carriers are compatible with each other.

Differential Scanning Calorimetry (DSC) Study

DSC thermograms (Figure 7) disclosed ND manifested a sharp endothermic peak at 124.6 °C with a narrow scale with an onset and endset temperature of 122 °C and 128 °C, respectively. The lyophilized formulation (F4) also showed a sharp endothermic peak at 123.08 ºC with onset and endset temperature of 122 to 127 °C indicating no interaction between ND and carriers used in the above formulation.

Pharmacokinetic Study

Table 5 represents selection of rabbit based on Latin Square crossover design for pharmacokinetic study of three samples. Serum ND concentration vs. time profile for aqueous suspension of ND, NE (F4), and lyophilized NE (F4) was presented in Figure 8. The PK parameters Cmax, Tmax, and AUC were determined. Higher Cmax was obtained for the NE (F4) and lyophilized NE (F4) comparison to the aqueous suspension of ND. The Tmax for the pure drug was 4 h, whereas Tmax in the case of NE (F4) and lyophilized NE (F4) was 1 and 1.5 h, respectively, indicating faster from nanoformulations. The AUC of the aqueous suspension of ND, optimized NE (F4) and lyophilized NE (F4) was 7351.21, 40786.67 and 38456.56 ng.hr/ml, respectively (Table 6). This indicates more than fivefold increase in bioavailability for NE and lyophilized NE.

Stability Study

The stability of NE (F4) and lyophilized F4 was evaluated by determining drug content, globule size, Zeta potential, and Q_{30} with a frequency of 1 month for 3 months at $25\pm2^0C/60\pm5\%$ RH (Table 7). In the case of NE (F4), globule size increased during the stability study (Significant at $P < 0.05$ level) whereas other parameters did not show any significant variation. In the case of lyophilized F4, no significant changes were observed for any of the parameters. Lyophilized F4 showed improved stability due to conversion of NE into solid form by lyophilization using trehalose as cryoprotectant.

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4. CONCLUSION

NE of ND with an optimum proportion of oil, surfactant, and co-surfactant has the potential to improve the *In-vitro* diffusion, whereas lyophilization ensured similar properties with improved stability. PK study for both NE (F4) and lyophilized (F4) in albino rabbit revealed an increase in oral bioavailability. Hence this approach may also be extended for other BCS class II drugs with poor oral bioavailability.

5. CONSENT FOR PUBLICATION

All the authors agreed to the publication of this manuscript.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Figure legends

Figure 1: Solubility of ND in oil (A), surfactant (B) and co-surfactant (C)

Figure 2: Ternary phase diagram for different ratios of oil and Smix

Figure 3: Contour plot and 3D plot showing the effect of different factor on responses

Figure 4: Overlay plot showing the design space and the optimised selected formulation

Figure 5: *In vitro* diffusion study pure drug ND, Nanoemulsion formulation (F4), Lyophilized nanoemulsion of F4 and marketed tablet of ND

Figure 6: FT-IR spectra for pure drug ND and lyophilized formulation of F4

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Figure 7: DSC thermogram of pure drug ND and lyophilized formulation of F4

Figure 8: Time Vs Serum ND concentration profile

Table 1: Composition of nanoemulsion formulations of Nimodipine as per BBD

Formulation	Triacetin (A)	Smix (B)	Water (C)	Drug Content (%)	Globule Size (nm)	Q30 (%)
F1	250	100	200	52.1	538	45.8
F2	250	100	100	58.5	522	41.3
F3	200	150	100	67.6	320	67.6
F4	**200**	**200**	**150**	**87.8**	**82**	**83.8**
F5	300	150	200	60.8	445	61.5
F6	300	150	100	63.8	422	51.7
F7	200	150	200	71.8	318	65.4
F8	250	150	150	66.7	342	62.5
F9	300	100	150	51.8	568	48.6
F10	250	150	150	66.2	334	62.2
F11	200	100	150	61.7	512	46.8
F12	250	150	150	64.8	348	61.8
F13	300	200	150	78.2	245	68.4
F14	250	150	150	64.4	345	61.4
F15	250	200	100	81.3	73	81.3
F16	250	150	150	65.8	338	58.8
F17	250	200	200	77.8	155	73.8

Table 2: Significance of different factors on different responses as per ANOVA

Source	Drug Content P Value	Globule Size P Value	Q30 P Value
Model	**< 0.0001**	**< 0.0001**	**< 0.0001**
Triacetin (A)	**0.0003**	**< 0.0001**	**0.0003**
Smix (B)	**< 0.0001**	**< 0.0001**	**< 0.0001**
Water (C)	0.1396	**0.0291**	0.4714
AB	0.9375	**0.0103**	**0.0027**
AC	0.0921	0.4433	**0.0201**
BC	0.4579	0.0691	**0.0201**
A^2	0.1547	**0.0035**	NA
B^2	**0.0155**	0.0221	NA
C^2	0.2964	0.7438	NA

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Table 3: Characterization of Nanoemulsions

Formulations	Dispersibility	Drug Content (%)	Globule Size (nm)	PDI	Zeta Potential (mV)
F4	Grade A	87.4 ± 2.4	76 ± 4.5	0.212 ± 0.03	-26.5 ± 0.8
Lyophilized F4	Grade A	86.5 ± 3.6	198 ± 5.7	0.245 ± 0.03	-26.6 ± 1.5

Mean ± SD, n = 6, Grade-A: Nanoemulsion formed in less than1 min and the appearance was clear

Table 4: In Vitro Diffusion related parameters for Nimodipine and its formulations

Formulations	DE_{30} (%)	MDT (Min)	Q_{30} min (%)
Pure drug ND	3.2 ± 0.02	63.12 ± 2.1	3.5 ± 0.025
F4	81.3 ± 2.5	18.81 ± 0.24	83.5 ± 3.65
Marketed tablet	15.4 ± 0.45	56.85 ± 1.2	17.3 ± 0.14
Lyophilized Formulation (F4)	78.3 ± 3.5	21.33 ± 0.25	79.3 ± 4.12

DE_{30} (%) is percent diffusion efficiency at 30 min. MDT is the mean diffusion time in min and Q_{30} min is the percentage of drug diffused in 30 min.

Table 5: Latin Square crossover design for Pharmacokinetic study of three samples in six male rabbits

Rabbits	Drug Product		
	Study period 1	Study period 2	Study period 3
1	Aqueous suspension of ND	F4	Lyophilized F4
2	F4	Lyophilized F4	Aqueous suspension of ND
3	Lyophilized F4	Aqueous suspension of ND	F4
4	Aqueous suspension of ND	Lyophilized F4	F4
5	Lyophilized F4	F4	Aqueous suspension of ND
6	F4	Aqueous suspension of ND	Lyophilized F4

Table 6: Pharmacokinetic Data

Parameters	Aqueous suspension of ND	F4	Lyophilized F4
Cmax (ng/mL)	1945.3 ± 76.4	3470.8 ± 103.4	3254.6 ± 143.4
Tmax (h)	4 ± 0.21	1 ± 0.13	1.5 ± 0.26
AUC (ng.h/L)	7351.21 ± 456.4	40786.67 ± 567.3	38456.56 ± 687.2

Mean ± SD, n = 6

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Table 7: Stability studies of Nanoemulsion F4 and Lyophilized F4

Time (months)	F4				Lyophilized F4			
	Drug content (%)	Globule Size (nm)	Q_{30}** (%)	ZP* (mV)	Drug content (%)	Globule Size (nm)	Q_{30}** (%)	ZP* (mV)
0	87.4 ± 2.4	76 ± 4.5	83.5 ± 6	-26.5 ± 0.8	86.5 ± 3.6	198 ± 5.7	79.3 ± 4	-26.6 ± 1.5
1	86.1 ± 1.5	96 ± 2.4	84.5 ± 6	-24.5 ± 1.2	87.5 ± 1.7	200 ± 3.2	76.1 ± 2	-25.6 ± 1.1
2	85.8 ± 2.1	112 ± 3.6	81.5 ± 6	-25.5 ± 0.8	83.4 ± 2.2	201 ± 3.1	78.9 ± 3	-26.2 ± 0.7
3	86.7 ± 1.1	118 ± 2.1	81.5 ± 6	-24.5 ± 0.9	84.5 ± 1.8	202 ± 2.1	78.5 ± 3	-26.3 ± 1.1
ANOVA at P < 0.05 level	No Sig	**Sig**	No Sig	No Sig	No Sig	No Sig	No Sig	No Sig

Mean ± SD, n = 6, *ZP = zeta potential, **Q_{30} min is the percentage of drug diffused in 30 min, No sig = no significant difference.