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RESPONSE SURFACE METHODOLOGY FOR AN IMPROVED NANOEMULSION OF IVACAFTOR & ITS OPTIMISATION FOR SOLUBILITY AND STABILITY

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ABSTRACT

A huge financial burden is imposed due to deaths caused by Inflammatory lung diseases. Treatment of inflammatory lung diseases can be targeted via the lungs because of their unique features. Inhalation therapy can target them with the help of inhaled therapy. This Research aims to present the wide-ranging role of nanoemulsions in treating inflammatory respiratory diseases by increasing the solubility of BCS class II drugs. Our target was to enhance the solubility of Ivacaftor, which belongs to a class of CFTR potentiators and a BCS class II drug. The solubility enhancement was to be achieved with the help of formulation into a nanoemulsion. Nanoemulsion has the advantage of being a thermodynamically stable formulation. We used Tween 20 as a surfactant and Transcutol-p as a co-surfactant. The region of the emulsion was identified after the construction of a pseudo-ternary phase diagram. The formulation was optimized using Design Expert 13, and the final preparation was evaluated for stability. The final formulation gave a globule size of 97.65±0.52 nm and a zeta potential of -1.75±0.46mV. The FTIR spectra showed very minimal changes keeping the peaks of the drug intact. We found the formulations were stable after a one-month stability study. Biological barriers in the human body can be overcome by utilizing nanoemulsions to achieve quicker onset of action and immediate relief. Inhaled nanocarriers pose a potential regarding translational studies and increase the scientific database for managing inflammatory lung diseases with reduced or no toxicity.

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Introduction

Cystic fibrosis is caused by a mutation in the gene located on chromosome 7's extended arm and is also an autosomal recessive disease. A protein regulates chloride ion transport channels across the cell membrane and intracellular membranes, known as cystic fibrosis transmembrane protein [1, 2]. It has been known to affect a considerable amount of the pediatric population and has been a cause of death amount infants and young children. It limits the eminence of life and endurance of the patient [3, 4]. Cystic fibrosis is a multisystemic disease known to cause insufficiency in the endocrine and exocrine functioning of the pancreas, bronchiectasis, liver dysfunction, and various other organs [3, 5]. This mutation in the CFTR gene impairs its function leading to increased secretion of sticky and thick mucus, causing obstruction in the airway and producing inflammatory responses in the pulmonary area [6, 7]. A distinct set of pathogens, including Burkholderia spp., Achromobacter spp., Stenophomonas maltophilia, several anaerobes, and non-tubercular mycobacteria, are a leading cause of chronic infections and mortality [8, 9].

Ivacaftor comes under the category of cystic fibrosis transmembrane potentiator (CFTR) [10, 11]. Ivacaftor works on opening the channel for improved transportation of chloride. Ivacaftor has a molecular weight of 392.5 Daltons and a half-life of 12 hours after administration of a single dose. The aqueous solubility of ivacaftor is as low as 0.05 µg/mL and has a pKa value of 11.08 [12]. The Cmax and AUC of Ivacaftor remain unaffected by the presence of food in the stomach. The bioavailability of this candidate varies from individual to individual. Therefore, has been categorized under BCS class IV. The pediatric dose

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of this drug is 150mg twice daily in the tablet dosage form [13, 14].
Nanoemulsion is considered an extensive option for the therapeutic approach under investigation to deliver pharmaceuticals under submicron size [15, 16]. For the systemic delivery of biologics, nanoemulsions are the most advanced drug delivery approach [17, 18]. A single-phase liquid is formed from two immiscible liquids with the application of surfactants to create a thermodynamically stable, isotropic system. The nanoemulsion droplet size was found in the range of 30-210 nm with a very narrow size distribution [19, 20]. To increase the solubility of the candidate and ultimately increase the availability of the drug at the site of action, along with reducing the dose, was our primary aim for categorizing nanoemulsion capsules of Ivacaftor for pediatric patients [21].
This study is focused on the delivery of nanoemulsion of IVF in the form of a capsule. The aim was to formulate, evaluate, characterize, and optimize the nanoemulsion loaded with IVF with the help of Design expert 13, to increase the solubility of IVF with the help of size reduction and enhance its absorption via the oral route and increase the bioavailability of this candidate. The surfactant and co-surfactant were selected after a thorough screening of the drug's solubility into them. Various Preformulation parameters were considered, such as pH and drug-excipient compatibility by FTIR and DSC analysis [22, 23]. After formulation of nanosuspension loaded with IVF, it was loaded into capsules and subjected to dissolution studies. The final formulation was evaluated for droplet size, polydispersity index, transmittance, zeta potential, Drug content, and In-vitro drug release profiles plotted.

Materials and Methods

Materials

Ivacaftor was obtained from Alembic Pharmaceuticals Limited Vadodara as a gift sample. Transcutol was used as a co-surfactant, and Labrasol was used as a surfactant. Both were obtained as a gift sample from Gattefossé. Captex 100, Acrysol, Labrafil, Capmul, Sefsol, Capryol, and Miglyol were obtained from Corel Pharma Ltd. Tween 20, Tween 80, and Span used were of analytical grade.

Solubility Study

Selection of Oil, Surfactant, and co-surfactant was made based on the solubility of the drug in them in excess quantity. Several Oils (Labrafil, Capmul MCM, Captex-100, Sefsol) surfactants and co-surfactants (Transcutol-p, Acrysol, Labrasol, Capryol, Tween 20, Tween 80, Span 80) were evaluated for their maximum ability to solubilize the drug into them by employing shake flask method. For the screening of individual schemes, the single component was taken, and the drug was added into excess and put on a rotary shaker for 24 hours [24, 25]. The system was then adequately diluted with methanol and screened for drug concentration at 384.3 nm with the help of a UV-visible spectrophotometer (UV-Shimadzu Corporation, Tokyo, Japan) against a blank (Methanol). Triplicate data was carried out, and the mean was recorded [26, 27].

Construction of Pseudo-Ternary Phase Diagram

After a long exploration for finding the suitable oil, surfactant, and co-surfactant, A Pseudo-ternary phase diagram peak was constructed using Oil, Smix (surfactant: Cosurfactant), and water with the help of the titration method. We used Chemix software for Surfactant (Tween 20) and Cosurfactant (Transcutol-p), and water was used in volume ratios of 1:1, 1:2, and 2:1 [28]. The Smix ratio was chosen in the concentrations increasing for the surfactant concerning cosurfactant so that the formation of nanoemulsion can be studied in detail. Every phase diagram was constructed with Oil (Capmul) and Smix (Tween20: Transcutol-p) in the ratio from 1:9 to 9:1 in glass vials separately. To study the delineated boundaries precisely, 10 different combinations were tried out for the construction of the phase diagram [29].
With the help of the aqueous titration method, pseudo-ternary phase diagrams were developed. A variable concentration of 5-95% of the aqueous phase was kept at 5% intervals. As the system is thermodynamically stable, it helps in the easy scale-up of the process. A 5% aqueous phase was added to the Surfactant-cosurfactant mixture, and visual examinations were made regarding its flowability and transparency [30]. Similar calculations were made for the subsequent Oil and Smix ratios where one axis denoted the aqueous phase, the other one denoted the Oil phase, and the third one was to represent Smix. The pseudo-ternary phase diagram was plotted only indicating nanoemulsion points to avoid overcrowding, and only that area is of significance from the formulation point of view [31].

Preparation of IVF-Loaded Nanoemulsion

The nanoemulsion zone was identified phase diagram of pseudo ternary type, and the parts of Oil, surfactant, and cosurfactant were obtained to formulate the nanoemulsion formulation. A total of five formulations containing Ivacaftor at 50mg/ml were planned and formulated. A mixture of drug and co-surfactant was prepared, subsequently adding the surfactant. The prepared mixture was introduced into the Oil fraction, and it was subjected to mixing on a vortex mixer to obtain a clear solution. Distilled water was introduced with the help of a syringe into the prepared mixture of surfactant, cosurfactant, and oil. The final solution was homogenized using a high-speed homogenizer set at 10000 rpm for 30 minutes. A clear formulation of nanoemulsion was prepared and visually evaluated for turbidity and saved for further research [32, 33].

Characterization of Nanoemulsion

Viscosity Measurement

The viscosity of the formulation was tested without dilution with the help of a digital viscometer (Brookfield viscometer, model LVDV-2+ Pro) to confirm the emulsion type. A W/O emulsion can be identified with the formulation having higher viscosity and vice versa for the O/W emulsion.

Zeta Potential, Droplet Size, and Polydispersity Index

The physical stability of the nanoemulsion depends largely upon the zeta potential of the formulation. There is a chance that surface charge may have developed during the preparation of the nanoemulsion. When the surface group becomes ionized, or we can see the ions are absorbed by the molecule, a potential is developed on the molecule known as zeta potential. The intermediate surroundings, as well as the surface charge of the molecule, also affect the charge that is acquired on the molecule's surface. The generated potential on the surface of the molecule loses its energy as we move away from the surface. Zeta potential helps in quantifying the speed of molecules in the suspension medium. Evaluation of nanoemulsion was done for Droplet size, zeta potential, and polydispersity index on Zetasizer Nano ZS (Malvern Instruments, Malvern UK) [34, 35].

Dilution and Dye Solubility Test

To confirm the nature of the emulsion, dilution, and tests are performed. If the formulation is of O/W type, then, upon dilution with water in the ratio of 1:10 or 1:100, it will not show any change; as water is in the continuous phase, it will remain stable. If we add in an O/W type of emulsion, it will break due to incompatibility. Similarly, in the dye test, a water-soluble dye will show colored droplets if the emulsion is W/O type and vice versa in the case of an oil-soluble dye [36, 37].

Percentage Transmittance

IVF-loaded nanoemulsion was subjected to % transmittance determination by UV-Vis Spectrophotometer. One liter of deionized water was used to dilute 1 ml of nanoemulsion, and it was observed by UV-Vis Spectrophotometer at 384.3 nm [38, 39].

Content of Drug

For the determination of drug content, 50 mg of Ivacaftor was dispersed in 100 ml of methanol with agitation. A definite part of the formulation was taken and again put into methanol for dilution. The formulation was put for examination in a UV-Vis spectrophotometer and checked for absorbance at 384. Nm.

Centrifugation

The centrifugation method is used to calculate the stability of the formulation for its physical stability. The prepared formulation was put for centrifuge at 3000 rpm. The nanoemulsion was then evaluated for turbidity, phase segregation, and transparency.

FTIR

To study any kind of interaction between the excipients and the drug, Fourier transform infrared spectroscopy is utilized. An FTIR Spectrum analysis was performed on the optimized batch, which was compared with the drug and excipient FTIR spectra. Any kind of peak fluctuation was considered in each spectrum.

In-vitro Dissolution

In-vitro dissolution studies were carried out using the dialysis bag release method. A dialysis bag of suitable pore size was selected and tied with thread from one end firmly so that any leakage could be prevented.

Results and Discussion

Solubility Analysis of the Drug in Oil

The drug solubility was examined in a few oils such as Labrafil, Capmul MCM, Captex-100 & Sefsol. The analysis revealed that the drug was most soluble in Capmul MCM.

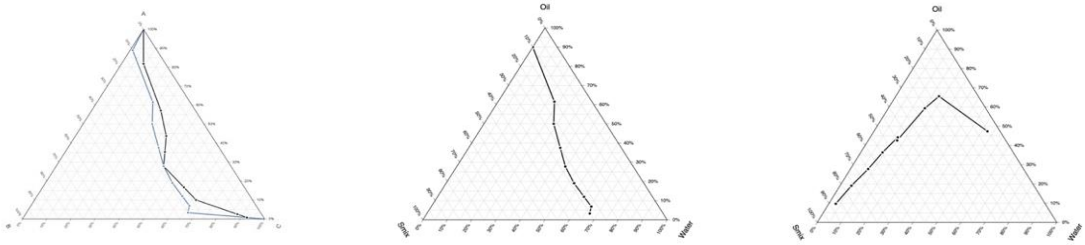
Surfactant and Co-Surfactant Screening

For the solubility study of the drug in surfactant and co-surfactant, a similar method was used as that was used in the solubility study of oils in Transcutol-p, Acrysol, Labrasol, Capryol, Tween 20, Tween 80, & Span 80. This study showed that the drug was highly soluble in Transcutol, which was used as a co-surfactant, and Tween 20, which was used as a surfactant.

Determination of Emulsion Region

By using the water-titration method, we plotted a pseudo-ternary phase diagram so that a stable nano emulsion in a perfect concentration scope could be prepared. The plot was constructed using Tween 20 as a surfactant and Transcutol as a co-surfactant in a ratio of 1:1. 1:2 and 2:1. It can be observed that the nanoemulsion zone was produced **(Figure 1)**. The broad area of the 1:1 plot was chosen, and six formulations were prepared arbitrarily from that area so that he t best batch could be selected from that. The HLB value of Tween 20 is higher than Transcutol. As we see an increase in the percentage of Tween

20, the assimilation of water also rises, but we observe a decrease in the solubility of the drug. Therefore we chose a 1:1 ratio of formulation for further investigation.



a) b) c)

Figure 1. Pseudo ternary phase diagram for (a)1:1, (b)1:2, and (c)2:1 oil and surfactant ratio. *For Figures 1a and 1b are on the right side of the line. They are on the right side of the bar, representing the clear nanoemulsion region, and for Figure 1c, they are above the line, representing the clear nanoemulsion region.

Formulation Table

A total of six nanoemulsion batches were prepared using the trial-and-error technique after getting the desired area in the ternary phase diagram in the ratio of 1:1 for the Smix. The composition table is summarized in **(Table 1)**.

Optimization of Nanoemulsion Using Factorial Design

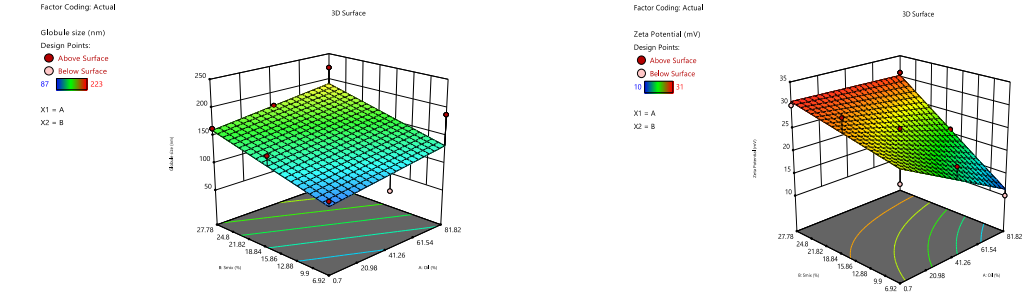
A factorial design was used for the determination of the influence of two factors, namely Oil (A) and Smix (B), and the result of their interactions on the responses over particle size and zeta potential. The matrix of the factorial design was prepared **(Table 1)**. The results identified which factors and their interactions have a significant influence over responses. A greater slope is an indication of greater influence over the variables in the system **(Figure 2a)**. The level of statistical significance for each factor and their effect were determined **(Table 1)**. In the optimised formulation the observed values for the evaluation parameters of oil(%), Smix(%), water(%), Transmittance (%), Drug Content(%), Zeta potential, and globule size was found to be 39.68%, 19.45%, 40.87%, 95.76±0.42%, 97.54±0.19%, -1.75±0.46 mV, and 97.65±0.52 nm respectively.

Table 1. Formulation optimization using factorial design

S.no.	Batch	Oil (ml)	Smix (ml)	Particle Size (nm)	Zeta Potential (mV)
1	F1	-1	-1	109.34 ± 0.3	22.6 ± 0.4
2	F2	-1	0	148.45 ± 0.5	31.8 ± 0.8
3	F3	-1	1	163.34 ± 0.3	30.5 ± 0.9
4	F4	0	-1	87.67 ± 0.7	21.4 ± 0.2
5	F5	0	0	118.45 ± 0.2	25.2 ± 1.3
6	F6	0	1	176.56 ± 1.1	29.8 ± 1.1
7	F7	1	-1	188.45 ± 1.3	10.7 ± 0.4
8	F8	1	0	104.45 ± 0.9	21.1 ± 0.6
9	F9	1	1	223.23 ± 0.5	31.5 ± 0.8
Variables level		**Low (-1)**		**Medium (0)**	**High (1)**
X1		0.78		41.26	81.82
X2		6.92		17.35	27.78

Zeta Potential and Size of the Globule

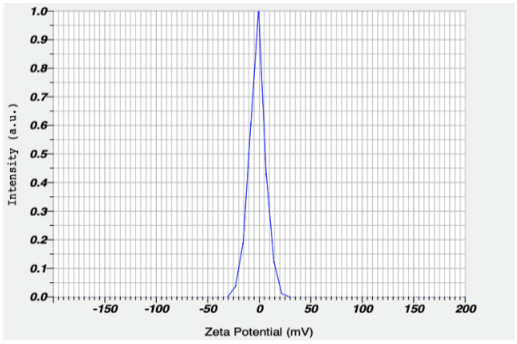
Figure 2c represents the mean globule size for the optimized batch at 97.65±0.52 nm with a low index of polydispersity. This concludes that globules were spread entirely in a very narrow range. The nanoemulsion range was confirmed by the observed globule size, which was below 100 nm. The motion of globules inside an electric field is measured with the help of surface charge. It can be seen in **Figure 2b** that the observed zeta potential was -1.75±0.46. The negativity of zeta potential maintains the stability of the formulation. Globule affinity can also be identified with the help of zeta potential.



Globule size Y1 = 146.92 + 15.83 A + 29.67 B

Zeta Potential Y2 = 24.44 – 3.50 A + 6.17 B + 3.25 AB

a)



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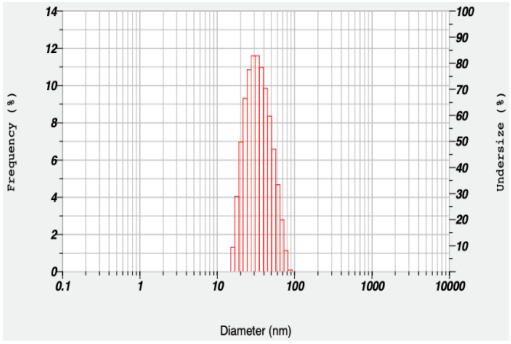

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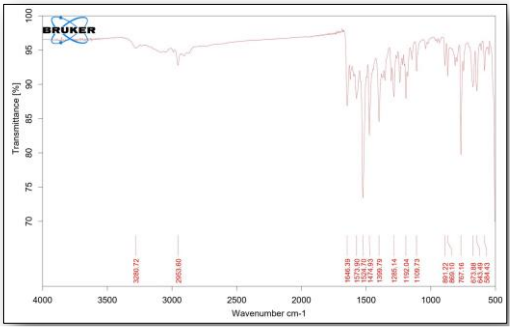

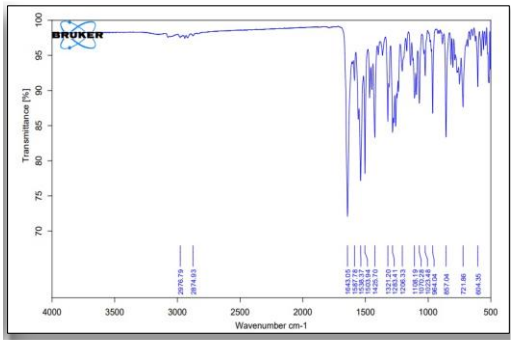

d)

Figure 2. (a) Optimization of Nanoemulsion using Design Expert 13, (b) Zeta potential for the optimized nanoemulsion, (c) Globule size for the optimized nanoemulsion, (d) FTIR spectra of pure drug and optimized formulation.

Appearance, % Transmittance, and Drug Content

All the formulations appeared clear. Drug content was in the range of 93.65±0.51 to 97.54±0.19, and % transmittance was observed from 90.98±0.56 to 95.76±0.42. The NE1 represented the best drug content with good % transmittance.

Centrifugation

The formulations were put to centrifugation for 1 hour at 3000 rpm, and no sign of precipitate formation or separation of phase was observed. This concluded that the system was a nanoemulsion **(Table 2)**.

pH Measurement

The optimized nanoemulsion had a pH of 6.45±0.21, which is considered for oral delivery **(Table 2)**.

Determination of Viscosity

A digital viscometer was used for the measurement of viscosity. Viscosity was observed at 83.73 ± 0.72 mPas at 40 rpm. Spindle SPL 1 was used for this purpose **(Table 2)**.

Dye solubility and Dilutability Test

Diluted the optimized batch with water in the ratio of 1:20 and 1:100, but there was no evidence of phase separation. The dye solubility test confirmed that it was an O/W type of nanoemulsion **(Table 2)**.

Table 2. Physiochemical parameters for the optimized batch

Factors	Optimized batch (F1)
Viscosity (mPas)	83.73 ± 0.72
Centrifugation	No phase separation

pH	6.45±0.21
Durability	No phase separation
Dye solubility test	O/W

Fourier Transform Infrared Spectroscopy

FTIR plots were used to compare the pure drug and the optimized batch. The spectral analysis of IVF and the optimized product is shown in **Figure 2d**. Very few changes were observed in the FTIR spectra of the pure drug and the optimized formulation.

In-vitro Drug Release Study

The dissolution profile of the formulation after 45 hours is shown in **Figure 3**, the formulation of pure drug exhibited 34.98 ± 0.24% CSR, and the optimized formulation of nanoemulsion exhibited 98.75±0.49% CDR. The results depicted that the optimized formulation showed improved drug release than the pure formulation. This concluded that the solubility of the formulation had been enhanced **(Table 3)**.

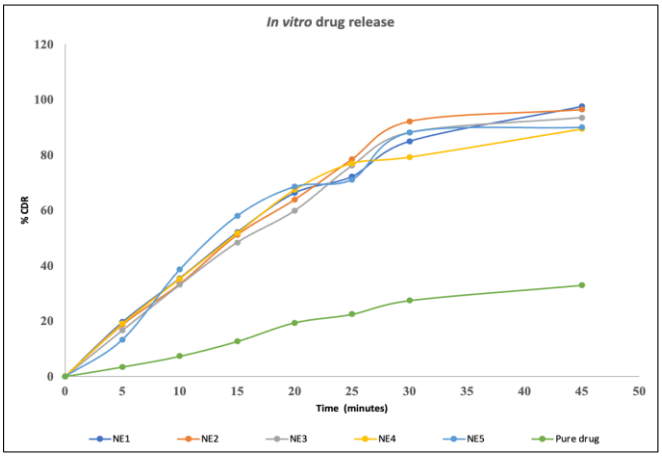

Figure 3. In-vitro dissolution profile F1-F5 and dispersion of pure drug

Table 3. Dissolution Profiles

Time (min)	F1	F2	F3	F4	F5	Pure Drug
0	0	0	0	0	0	0
5	19.64±0.28	18.72±0.14	16.68±0.25	19.08±0.15	13.36±0.24	3.45 ±0.32
10	35.52±0.34	33.48±0.27	33.12±0.16	35.33±0.23	38.65±0.34	7.32 ±0.85
15	52.13±0.45	51.21±0.38	48.44±0.28	51.76±0.25	58.04±0.27	12.67 ±0.53
20	66.35±0.76	63.95±0.17	59.88±0.36	67.45±0.45	68.56±0.13	19.43 ±0.57
25	72.25±0.78	78.53±0.29	76.14±0.42	77.05±0.07	71.15±0.28	22.48 ±0.53
30	84.99±0.64	92.19±0.19	88.13±0.38	79.27±0.32	88.13±0.23	27.45 ±0.29
45	98.75±0.49	96.44±0.24	93.49±0.26	89.42±0.27	89.98±0.18	34.58 ±0.24

Stability Analysis

A stability study for the formulation was accomplished for one month at 40°±2°C/75% RH. At this condition for stability % CDR and % drug content was determined. No major changes were observed in % drug content and % CDR. Stability studies are shown in **Table 4**. The nanoemulsion formulations were stable during the stipulated time.

Table 4. Stability Data

Formulation	**One Month Stability**	
	% Drug content (40°±2°C/75% RH)	**% CDR (40°±2°C/75% RH)**
F1	95.56 ± 0.15	97.63 ± 0.23
F2	96.76 ± 0.34	96.35 ± 0.18
F3	95.73 ± 0.54	95.29 ± 0.16
F4	94.54 ± 0.34	90.14 ± 0.32
F5	94.55 ± 0.63	59.98 ± 0.42

Conclusion

Nanoemulsion was prepared in several different ratios with Smix with the help of the Pseudo ternary phase. After identification of the emulsification region, we prepared six individual formulations having Tween 20, Transcutol, and Capmul MCM as

Surfactant, co-surfactant, and oil. This formulation exhibited a zeta potential of -15.75±0.46 mV globule size of 97.65±0.52 nm. % CDR was determined after In-vitro studies which were found to be 96.63±0.23 till 45 minutes which was found to be better than the dispersion of pure drug. Prepared formulations were found to be stable after one-month stability studies were executed. This study reveals that nanoemulsion can be a capable approach for the solubility improvement of Ivacaftor.

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Conflict of interest: None

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Ethics statement: The paper reflects the author's own research and analysis in a truthful and complete manner.

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