

FORMULATION AND EVALUATION OF DOLUTEGRAVIR SODIUM NANOEMULSION FOR THE TREATMENT OF HIV

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ABSTRACT

The purpose of this research was to develop and evaluate the Nanoemulsion of Dolutegravir sodium. Nanoemulsion was made by the high-shear homogenization method. Materials and method: Oleic acid was used as the oil phase, Tween 80, and Acconon MC8-2 EP/NF (Smix; 1:1) were utilized as surfactant and co-surfactant respectively while distilled water was employed as an aqueous phase. The formulations were evaluated based on globule size, zeta potential, pH, viscosity, percentage transmittance, centrifugation, dye test, dilutability test, and Fourier transform spectroscopy. Furthermore, *in vitro* drug release studies and stability studies were also conducted. NE1 has shown the least particle size of 96.71 nm, viscosity of 86.4 cp, and maximum drug release of 97.55 % CDR in 45 hrs compared with pure drug (32.98% CDR in 45 hrs). The stability study confirmed no changes in formulations at 1 month at $40^{\circ} \pm 2^{\circ}\text{C}$ and $75\% \pm 5\%$. The findings suggest that nanoemulsions might be an effective vehicle for the oral administration of Dolutegravir sodium for the treatment of HIV.

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Introduction

Dolutegravir sodium (DTGS) is a second-generation integrase strand transfer inhibitor that blocks HIV viral DNA integration into host DNA, which is a necessary stage in viral reproduction. The postulated mechanism of action is the potential of DTGS to precipitate enzyme-linked cations, limiting viral DNA insertion in the host gene. The DTGS is preferred over other integrase inhibitors because it has relatively lesser resistance in the body. It has a bioavailability of 34%. Its half-life is 14 hrs. DTGS belongs to Bio-Pharmaceutical Classification System II drugs. The drugs which belong to BCS II have low aqueous solubility and high permeability [1-5].

Drug efficacy is significantly influenced by solubility, and the drug dissolution profile is influenced by the dissociation constant, pH, and the GI fluids' buffer capacity of the gastrointestinal fluid. If somehow the oral drug delivery is restricted for administrating drugs with unsupportable properties then the strategy consists in creating customized dosage forms. Consequently, plenty of focus has been placed in recent decades by scientists on increasing the solubility of those poorly aqueous soluble drugs, by employing any of the different drug delivery approaches such as hot melt extrusion, supercritical fluid technology, micronization, nanosization, salt formation, solid dispersion, spray drying technique, complexation, co-crystallization, prodrug formation, and lipid-based drug delivery including Nanoemulsion (NE) [6-10].

Nanoemulsion is an isotropic mixture of two immiscible liquids developed by using surfactants having globule sizes below 100 nm. Nanoemulsion possesses longer self-life and greater stability. It is believed to be one of the potential techniques to improve the drug release time and thus bioavailability of scantily aqueous soluble drugs [11-13].

A proper understanding of the oils, surfactants, and co-surfactants required to prepare nanoemulsion is necessary to select excipients that will result in robust, stable nanoemulsion. The solubility and entrapment efficiency of drugs as well as the biological fate and globule size of nanoemulsion are all significantly influenced by the physicochemical characteristics of

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the oils. The surfactants that have a higher potential for dissolving the solid substance and the capability to reduce the interfacial tension need to be chosen for the development of the nanoemulsion. A cosurfactant is necessary since it is difficult for a solitary surfactant to provide temporary negative interfacial tension [14-18].

Co-surfactant assists in creating a liquid interfacial layer that influences the interfacial bending force by lowering it and allows the formation of an interfacial film with adequate flexibility to get the various shapes required to create nanoemulsions throughout a wide range of compositions. As a result, the optimal choice of nanoemulsion components is necessary for scaling [19-21].

In the current investigation, DTGS is made into a nanoemulsion formulation to enhance its dissolution profile.

Materials and Methods

Materials

Dolutegravir sodium was obtained as a gift sample from Emcure Pharmaceutical Pvt. Ltd., Ahmedabad, India. Acconon MC8, Capmul MCM C8, Captex 100, Captex 200, and Captex 300 were obtained as gift samples from Abitec corporation India. Lbabrafil 1944 cs, Labrasol, Cremophore EL were obtained as gift samples from Gattefosse India Pvt Ltd. All other chemicals and reagents were of analytical grade.

Solubility Studies

Oil, surfactant, and cosurfactant were chosen depending on their potential to dissolve a large amount of the drug. Various oils (Capmul MCM C8, Lbabrafil 1944 cs, Captex 100, Captex 200, Captex 300, Arachis oil, Sunflower oil, Oleic acid, Sefsol 228, Miglyol 840), surfactants and cosurfactants (Labrasol, Tween 20, Tween 60, Tween 80, Span 20, Span 80 Span 40, and Acconon MC8 Cremophore EL) were evaluated for solubility using the shake-flask method. After that, the individual system was spun for 10 minutes at 10,000 rpm in a high-speed centrifuge. After adequate dilution with methanol, the drug concentration of each system was measured at 260 nm using a UV visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) against a blank (methanol). The investigation was carried out in triplicate, and the mean data were recorded [22, 23].

Construction of Pseudo-Ternary Phase Diagram

According to the findings of an exploratory solubility study, the ternary phase diagram's peak was constructed using constituents including oil, surfactant, and co-surfactant. By using CHEMIX software, the pseudo ternary phase diagrams for all mixtures were created without the addition of drugs to determine the ideal concentration of oil, surfactant, and co-surfactant for preparing nanoemulsions as well as to identify the presence of the optimum emulsifying region. The ratio of chosen surfactants:co-surfactants (Smix) was prepared by combining them in different volume proportions (1:1, 1:2, 2:1). The construction of each phase diagram involved combining oil with precise Smix in nine ratios [24, 25].

Preparation of Dolutegravir Sodium-Loaded Nanoemulsion

The emulsifying zone was recognized after examining the pseudo-ternary phase diagram, and the proportions of surfactant, co-surfactant, and oil were determined to prepare the formulations. Five formulations containing DTGS at a strength of 10 mg/ml were prepared. The drug was first mixed in the co-surfactant, and then a sufficient quantity of surfactant was added. The oil fraction was subsequently introduced to the mixture after adequate mixing. All of the ingredients were mixed thoroughly together utilizing a vortex mixer until a clear solution was formed. The distilled water is injected into the above solution. The mixture is subjected to high-speed homogenization at 10000 rpm for 30 minutes. At room temperature, the nanoemulsion remained clear and was saved for further research [26, 27].

Characterization of Nanoemulsion

Appearance

All formulations were examined for transparency and indications of turbidity. Furthermore, all batches were evaluated with bare eyes against a white and black backdrop to look for any precipitation indications. The testing was carried out as per US Pharmacopeia (USP) [28].

Droplet Size and Polydispersibility Index

The globule size and polydispersity index (PDI) of the synthesized 5 formulations (F1-F5) comprising the DTGS were evaluated utilizing Zetasizer (Malvern® Instruments Limited, Worcestershire, UK) [29].

% Transmittance (% T)

% transmittance of the DTGS-loaded nanoemulsion was determined using a UV-Vis spectrophotometer. 1 mL of nanoemulsion was mixed with 1000 mL of deionized water. % transmittance of the diluted formulation was measured using a UV- spectrophotometer at 260 nm. The test was carried out in triplicate [30].

Dilutability and Dye Solubility Test

The dye test was carried out to determine if the nanoemulsion was oil in water (o/w) or water in oil (w/o). The emulsion surface was sprayed with a water-soluble color. When the nanoemulsion is o/w, the whole nanoemulsion is observed to be colored, and when it is w/o, small dots of dye are visible upon microscopic inspection. In the dilutability test, nanoemulsion is diluted with distilled water in 1:10 and 1:100 ratios to look for any indications of dissociation [31].

Viscosity Measurement

Measuring the viscosity of nanoemulsion with a digital viscometer (Brookfield viscometer) verifies the kind of emulsion. Larger viscosity indicates that the emulsion is w/o, whereas lower viscosity indicates that the emulsion is o/w [32].

Zeta Potential

Zeta potential is a crucial measure for observing the physical stability of nanoemulsion. Surface charge development on globules may be possible in Nanoemulsion. The cause for zeta potential development is that a surface group becomes ionized or ions adsorb. The acquired charge is also affected by the globules' immediate surroundings as well as the composition of a surface. The surface charge of the globules generates a potential that is relatively strong near the surface and reduces as the distance rises. The motion of the globules in the electric field may be quantified by zeta potential by calculating their speed in the suspending medium. Nanoemulsion was evaluated for zeta potential by Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) [33].

Drug Content

A nanoemulsion containing 10 mg of DTGS was mixed in 100 mL methanol. The solution was well agitated. An aliquot of the solution is taken, diluted with methanol, and absorbance at 260 nm was analyzed using a UV-spectrophotometer [34].

Centrifugation

The objective of centrifugation was to assess NE's physical stability. For 60 minutes, centrifugation was performed at 3,000 rpm. NE was then tested for transparency, phase segregation, and turbidity [35].

Fourier Transform Infrared Spectroscopy (FTIR)

To determine the stability of formulation and whether or not there is an interaction between the drug and the excipients being utilized, an FTIR examination of the optimized batch was carried out. The FTIR spectra of DTGS and the spectra of the excipients were compared. DTGS peak loss or peak fluctuation in each spectrum was taken into account [36].

In Vitro Drug Release

The dialysis sac technique was used to evaluate *in vitro* drug release study. The dialysis bag with the required pore size is tied with thread firmly from one end to prevent leaking of the formulation. One ml of nanoemulsion containing 10 mg of the drug was poured into a dialysis bag. The dialysis bag was placed in a glass beaker filled with 100 mL of pH 7.4 phosphate buffer. The beaker was put on a magnetic stirrer and the temperature was maintained at 37 °C. A magnetic bead stirrer controlled the agitation of the beaker, and aluminum foil was used to protect the beaker to prevent solvent loss throughout the experiment [37, 38].

Results and Discussion

Solubility Study of Drug in Oil

From the solubility study of the drug in various oil like Oleic acid, Capmul MCM EP, Labrafac PG, Captex 200P, Capmul MCM PG 8 NF, Captex 355, Capmul MCM C8, and Miglyol 812. In this study, Oleic acid demonstrated a profound solubility of 34 ± 0.87 mg/ml which was chosen for further studies. The results are depicted in **Figure 1**.

Screening of Surfactants and Co-Surfactants

Solubility of the drug in surfactants (Tween 20, Tween 80, Cremophor EL, span 80, and span 20) was carried out the same way as the above method for oil. From the different surfactants, Tween 80 and Acconon MC 8-2 EP were selected as they show the highest solubility 23.58 ± 0.18 and 34.56 ± 3.18 respectively. The results of the solubility of the drug in various surfactants are depicted in **Figure 1**.

Pseudo-Ternary Phase Diagram

A pseudo ternary phase diagram was generated employing the water titration approach to provide a robust nano-emulsion with a precise concentration gamut. To construct the pseudo ternary plot, Tween 80 was taken as the surfactant and Acconon MC8-2 as the co-surfactant in the ratios of 1:1, 2:1, and 1:2. Oil, S mix, and water. **Figure 1** depicts the nanoemulsion zone produced. A broader 1:1 area was chosen, and five formulations were chosen at arbitrary out of that area to create the best batch. Tween 80 has high HLB value than acconon MC8-2. As the percentage of Tween 80 rises, so does the assimilation of water, but the drug's solubility lowers, hence 1:1 was chosen for subsequent investigation.

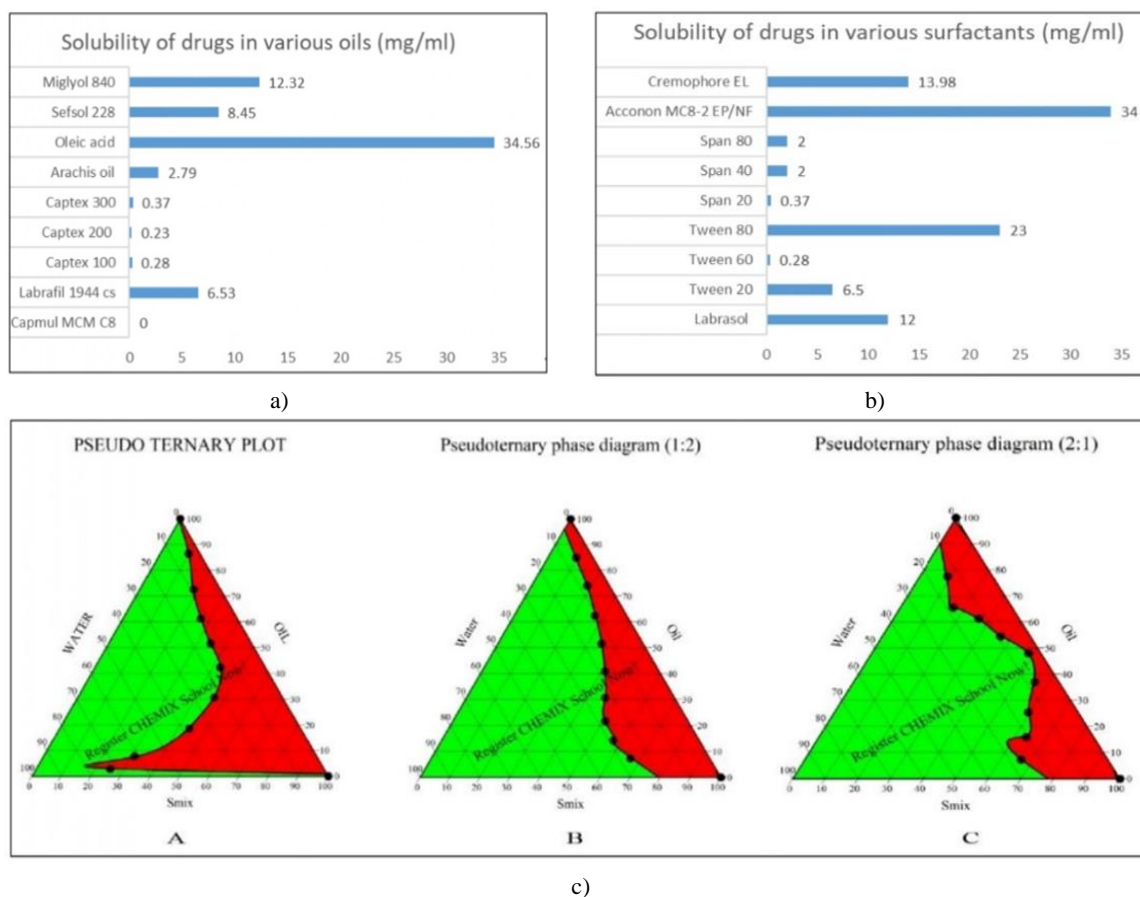


Figure 1. a) Solubility of the drug in various oils, b) Solubility of the drug in various surfactants, c) Pseudo ternary phase diagram of Smix ratio (A) 1:1 (B) 1:2 (C) 2:1

Formulation Table

Five distinct batches of nanoemulsion were made using the trial and error technique using the chosen ternary phase diagram 1:1 ratio of Smix. The composition is summarised in **Table 1**.

Table 1. Composition of formulations and results of % transmittance and drug content

Batch no.	Drug Mg/ml	Oil %v/v	Smix %v/v	Water %v/v	% Transmittance	% Drug content
NE 1	10	10	80	10	89.67±0.32	92.54±0.19
NE 2	10	10	60	30	73.67±0.93	80.56±0.24
NE 3	10	10	70	20	72.63±0.74	83.19±0.78
NE 4	10	10	50	40	71.35±0.15	86.87±0.83
NE 5	10	20	70	10	79.89±0.46	81.43±0.23

Globule Size and Zeta Potential

The mean globule size of the optimum batch is depicted in **Figure 2**, which is 96.71 nm with a low polydispersibility index, demonstrating that globules are spread across the entire system in a narrow range. The obtained globule size was < 100 nm which confirms the nanoemulsion range. The surface charge on the particle measures the motion of a globule inside an electric field. **Figure 2** reveals that the optimized batch has a zeta potential of -15.2 mv. Stability can be governed by the negativity of zeta potential, the more the negative value increases the stability. The zeta potential is essential for evaluating the stability of dispersions. The zeta potential can be used to identify the affiliation of globules in an electric field. Nearly all hydrophilic colloids are electronegative, with a zeta potential ranging from -13 to -30 millivolts.

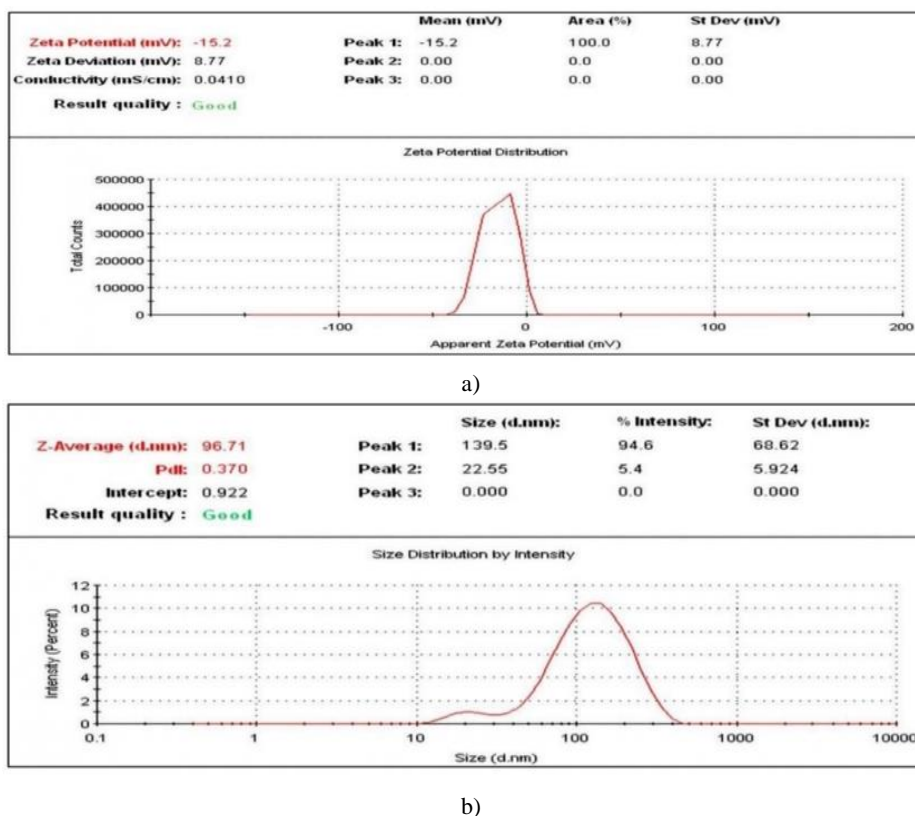


Figure 2. a) Zeta Potential of optimized Nanoemulsion NE-1, b) Globule size of optimized Nanoemulsion NE-1

Appearance, % Transmittance, and Drug Content

The appearance of all the formulations was found to be clear. Drug content was observed from 81.43 ± 0.23 to 92.54 ± 0.19 and % transmittance was found in the range of 71.35 ± 0.15 to 89.67 ± 0.32 which is shown in **Table 1**. The NE 1 depicted a good % transmittance with the highest drug content.

Centrifugation

After 1 hr of centrifugation at 2000 rpm, there was no evidence of phase separation/precipitation in NE 1. It was determined that the system is Nanoemulsion. The result is shown in **Table 2**.

pH Measurement

The pH of the optimized NE was discovered to be 6.45 ± 0.38 , making it appropriate for oral delivery. The result is depicted in **Table 2**.

Viscosity Determination

The digital viscometer was used to measure the viscosity of the optimized nanoemulsion. LMDV-60. Viscosity was found to be 86.4 ± 0.78 mPas at 30 RPM utilizing spindle SPL 1. The result is depicted in **Table 2**.

Dilutability and Dye Solubility Test

There was no trace of phase separation after dilution of the optimized batch with water in the ratios of 1:10 and 1:100. From the dye solubility test, it was determined that Nanoemulsion was O/W type. The result is shown in **Table 2**.

Table 2. Physicochemical parameters of optimized batch

Parameters	Optimized batch (NE 1)
Viscosity (mPas)	86.4 ± 0.78
Centrifugation	No phase separation
pH	6.45 ± 0.38
Dilutability	No phase separation
Dye solubility test	O/W

Mean \pm S.D *n = 3

Fourier Transform Infrared Spectroscopy

The FTIR investigation of the pure drug and the optimized batch was compared. The spectra of DTGS and the optimized product are shown in **Figure 3**. Results indicate that minor change was observed in the FTIR spectra of optimized formulation as related to FTIR spectra of pure drug.

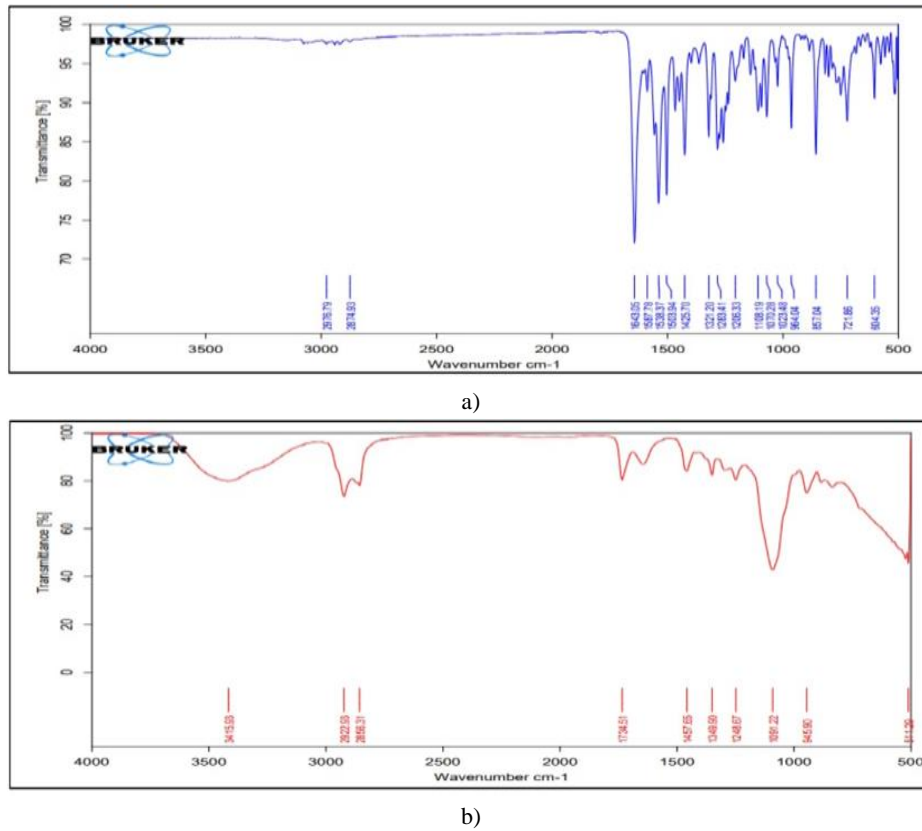


Figure 3. FTIR spectrum of a) Doltegravir sodium, b) Optimized nanoemulsion

In Vitro Drug Release Study

According to **Figure 4**, which depicts the dissolution profile after 45 hours, pure drug exhibited $32.98 \pm 0.4\%$ CDR, and optimized NE showed $97.55 \pm 0.2\%$ CDR. Based on the results, it was found that NE exhibits improved drug release than pure drugs, which suggests that solubility has been enhanced.

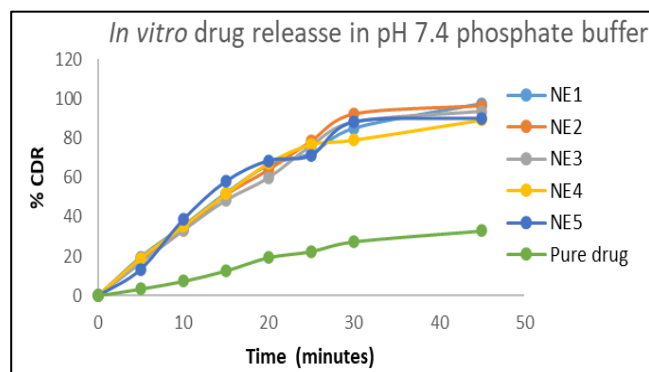


Figure 4. *In vitro* drug release NE1-NE5 and pure drug dispersion

Stability Study

The stability study of formulations was accomplished for up to 1 month at $40^{\circ} \pm 2^{\circ}\text{C}$ and $75\% \pm 5\%$ RH. % CDR as well as % drug content was determined. One month stability study demonstrated no major changes in % CDR and % drug content. The results of the stability study are shown in **Table 3**. Hence NE formulations were found to be stable.

Table 3. Stability study of Nanoemulsion

Formulation	% Drug content	% CDR
NE1	$94.98 \pm 0.3\%$	$96.45 \pm 0.2\%$

NE2	95.45 ±0.5 %	95.68 ± 0.1%
NE3	96.85 ± 0.1%	94.56 ± 0.7%
NE4	93.74 ± 0.4%	88.32 ± 0.5%
NE5	93.65 ± 0.5%	86.69± 0.8 %

Conclusion

NE was developed by employing a pseudo-ternary phase diagram for different S mix ratios. Five distinct compositions were chosen from the emulsifying region of the pseudo-ternary phase diagram. Formulation containing oil oleic acid (10%), Smix tween 80: Acconon MC8-2 EP/NF (80%) was optimized, which has demonstrated globule size 96.71 nm and zeta potential -15.2 mv. *In vitro* drug release study revealed 97.55% CDR up to 45 mins which is much better than pure drug dispersion which has exhibited just 32±0.2% CDR up to 45 mins. One month stability study has shown the formulations were stable. Nanoemulsion can be a promising approach for the improvement of the solubility of Dolutegravir sodium.

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