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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 $40^0 \pm 2^0$C and 75% ± 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

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the biological fate and globule size of nanoemulsion are all significantly influenced by the physicochemical characteristics of 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., Ahemdabad, 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. Inthis 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.

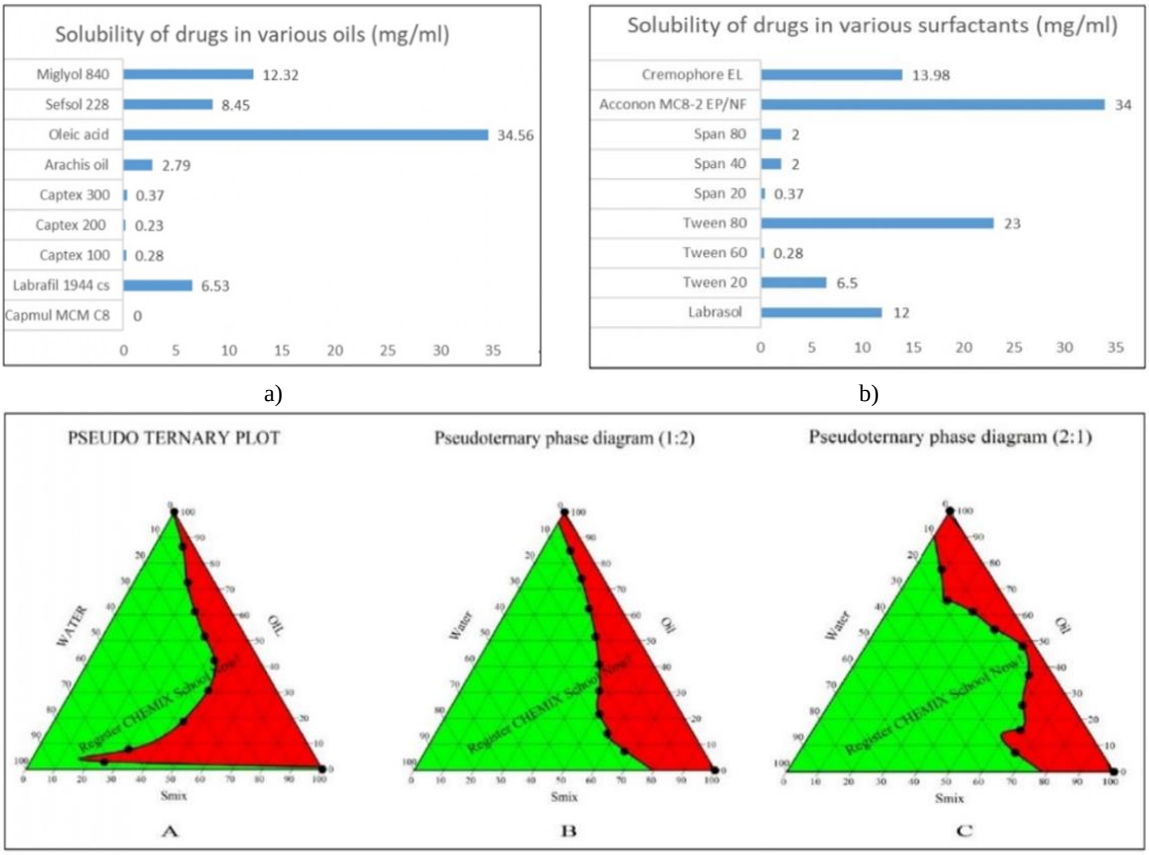


Figure 1. a) Solubility of the drug in various oils, b) Solubility of the drug in various surfactants, c) Pseudo ternary phase diagram of Smix ratio (A) 1:1 (B) 1:2 (C) 2:1

Formulation Table

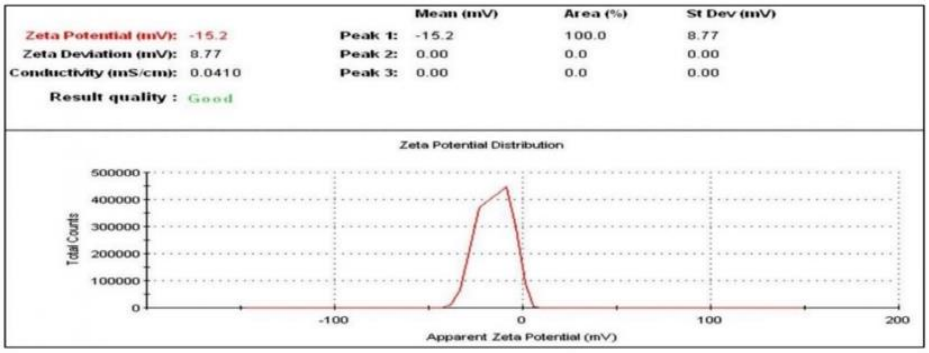
Five distinct batches of nanoemulsion were made using the trial and error technique using the chosen ternary phase diagram 1:1 ratio of Smix. The composition is summarised in **Table 1**.

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

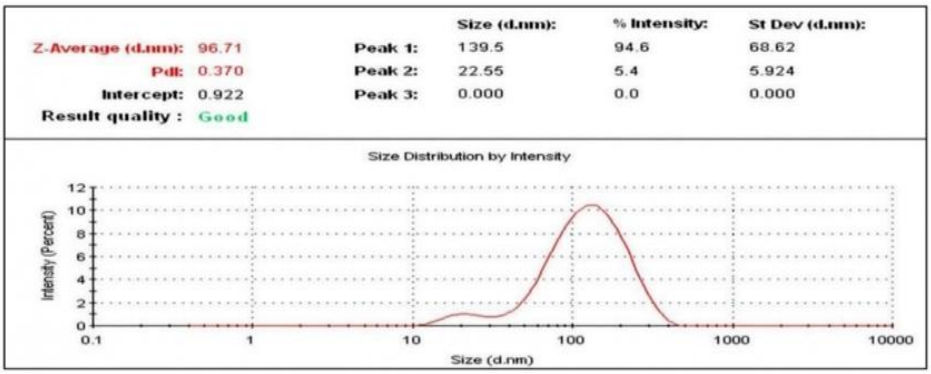
Batch no.	Drug Mg/ml	Oil %v/v	Smix %v/v	Water %v/v	% Transmittance	% Drug content
NE 1	10	10	80	10	89.67±0.32	92.54±0.19
NE 2	10	10	60	30	73.67±0.93	80.56±0.24
NE 3	10	10	70	20	72.63±0.74	83.19±0.78
NE 4	10	10	50	40	71.35±0.15	86.87±0.83
NE 5	10	20	70	10	79.89±0.46	81.43±0.23

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.



a)



b)

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**.

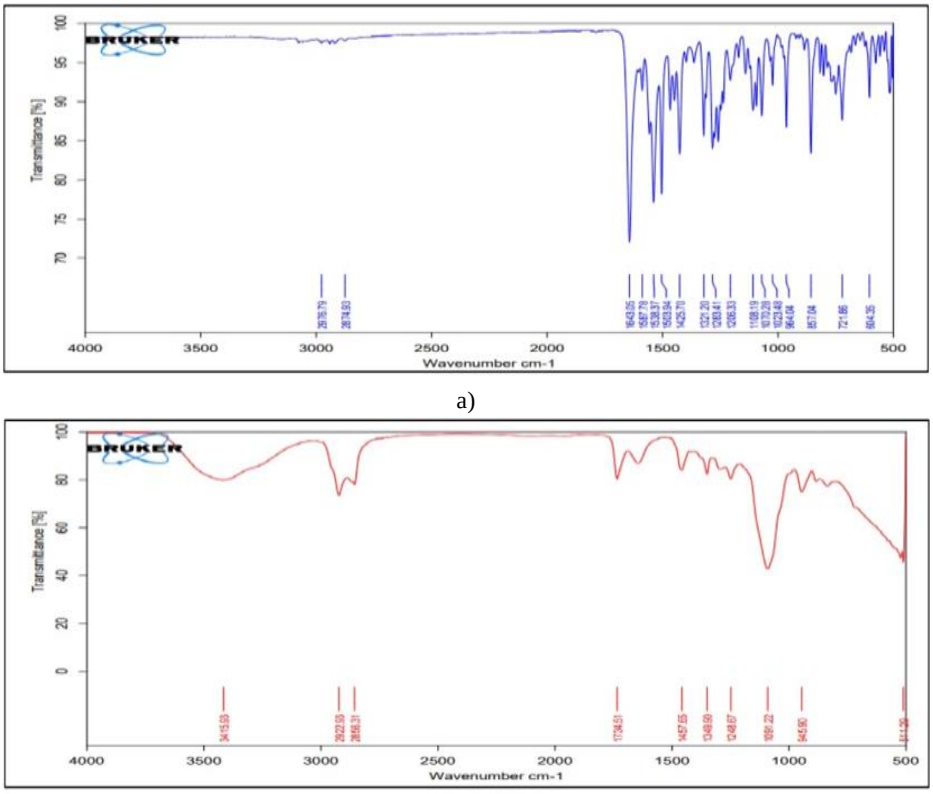
Table 2. Physicochemical parameters of optimized batch

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 ± 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.



a)

b)

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± 0.4% CDR, and optimized NE showed 97.55± 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.

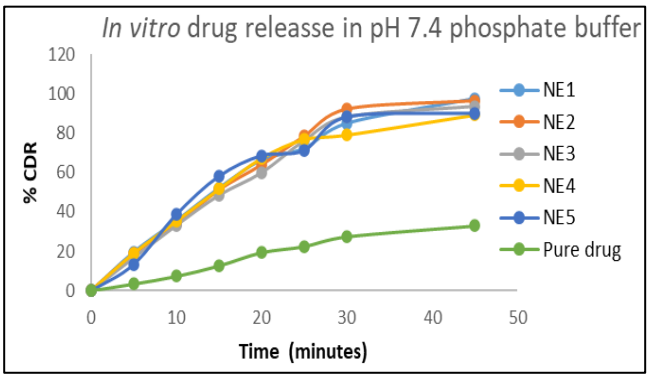


Figure 4. *In vitro* drug release NE1-NE5 and pure drug dispersion

Stability Study

The stability study of formulations was accomplished for up to 1 month at $40^0 \pm 2^0$C and 75% ± 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 ± 0.3%	96.45 ± 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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Conflict of interest: None

Financial support: None

Ethics statement: None

References

1. McCormack PL. Dolutegravir: a review of its use in the management of HIV-1 infection in adolescents and adults. Drugs. 2014;74(11):1241-52.
2. Kandel CE, Walmsley SL. Dolutegravir - a review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug Des Devel Ther. 2015;9:3547-55.
3. Wu G Abraham T, Saad N. Dolutegravir for the treatment of adult patients with HIV-1 infection. Expert Rev Anti Infect Ther. 2014;12(5):535-44. doi:10.1586/14787210.2014.907525
4. Sillman B, Bade AN, Dash PK, Bhargavan B, Kocher T, Mathews S, et al. Creation of a long-acting nanoformulated dolutegravir. Nat Commun. 2018;9(1):1-4.
5. Singh D, Bedi N, Tiwary AK. Enhancing solubility of poorly aqueous soluble drugs: critical appraisal of techniques. J Pharm Investig. 2018;48(5):509-26.
6. Jain H, Chella N. Methods to improve the solubility of therapeutical natural products: a review. Environ Chem Lett. 2021;19(1):111-21.
7. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. Int J Pharm. 2011;420(1):1-10.
8. Kumar R, Thakur AK, Chaudhari P, Banerjee N. Particle Size Reduction Techniques of Pharmaceutical Compounds for the Enhancement of Their Dissolution Rate and Bioavailability. J Pharm Innov. 2022;17(2):333-52.
9. Pathak K, Raghuvanshi S. Oral Bioavailability: Issues and Solutions via Nanoformulations. Clin Pharmacokinet. 2015;54(4):325-57.
10. Prajapati BG, Patel MM. Conventional and alternative pharmaceutical methods to improve oral bioavailability of lipophilic drugs. Asian J Pharm. 2007;1(1):1-8.
11. Santalices I, Vázquez-Vázquez C, Santander-Ortega MJ, Lozano V, Araújo F, Sarmento B, et al. A nanoemulsion/micelles mixed nanosystem for the oral administration of hydrophobically modified insulin. Drug Deliv Transl Res. 2021;11(2):524-45.
12. Jarzębski M, Fathordoobady F, Guo Y, Xu M, Singh A, Kitts DD, et al. Pea Protein for Hempseed Oil Nanoemulsion Stabilization. Molecules. 2019;24(23):4288.
13. Patel MR, Patel MH, Patel RB. Preparation and in vitro/ex vivo evaluation of nanoemulsion for transnasal delivery of paliperidone. Appl Nanosci. 2016;6(8):1095-104.
14. Panatieri LF, Brazil NT, Faber K, Medeiros-Neves B, von Poser GL, Rott MB, et al. Nanoemulsions Containing a Coumarin-Rich Extract from Pterocaulon balansae (Asteraceae) for the Treatment of Ocular Acanthamoeba Keratitis. AAPS Pharm Sci Tech. 2017;18(3):721-8.
15. Ma P, Zeng Q, Tai K, He X, Yao Y, Hong X, et al. Development of stable curcumin nanoemulsions: effects of emulsifier type and surfactant-to-oil ratios. J Food Sci Technol. 2018;55(9):3485-97.

16. Kassaee SN, Mahboobian MM. Besifloxacin-loaded ocular nanoemulsions: design, formulation and efficacy evaluation. Drug Deliv Transl Res. 2022;12(1):229-39.
17. Bhattacharya S, Prajapati BG. Formulation and optimization of celecoxib nanoemulgel. Asian J Pharm Clin Res. 2017;10(8):353-65.
18. Asgari HT, Es-high A, Karimi E. Anti-angiogenic, antibacterial, and antioxidant activities of nanoemulsions synthesized by Cuminum cyminum L. tinctures. J Food Meas Charact. 2021;15(4):3649-59.
19. Yadav P, Rastogi V, Verma A. Application of Box–Behnken design and desirability function in the development and optimization of self-nano emulsifying drug delivery system for enhanced dissolution of ezetimibe. Futur J Pharm Sci. 2020;6(1):1-20.
20. Jang JH, Jeong SH, Lee YB. Enhanced lymphatic delivery of methotrexate using w/o/w nanoemulsion: In vitro characterization and pharmacokinetic study. Pharmaceutics. 2020;12(10):1-18.
21. Md S, Alhakamy NA, Aldawsari HM, Husain M, Kotta S, Abdullah ST, et al. Formulation design, statistical optimization, and in vitro evaluation of a naringenin nanoemulsion to enhance apoptotic activity in a549 lung cancer cells. Pharmaceuticals. 2020;13(7):1-21.
22. Pavoni L, Perinelli DR, Bonacucina G, Cespi M, Palmieri GF. An overview of micro-and nanoemulsions as vehicles for essential oils: Formulation, preparation and stability. Nanomaterials. 2020;10(1):135.
23. Bhalerao H, Koteshwara K, Chandran S. Design, optimisation and evaluation of in situ gelling nanoemulsion formulations of brinzolamide. Drug Deliv Transl Res. 2020;10(2):529-47.
24. Karami Z, Khoshkam M, Hamidi M. Optimization of Olive Oil-Based Nanoemulsion Preparation for Intravenous Drug Delivery. Drug Res (Stuttg). 2019;69(5):256-64.
25. Kelmann RG, Colombo M, Nunes RJ, Simões CMO, Koester LS. Nanoemulsion-Loaded Hydrogels for Topical Administration of Pentyl Gallate. AAPS Pharm Sci Tech. 2018;19(6):2672-8.
26. Moradi S, Barati A, Salehi E, Tonelli AE, Hamedi H. Preparation and characterization of chitosan-based hydrogels containing cyclodextrin inclusion compounds or nanoemulsions of thyme oil. Polym Int. 2019;68(11):1891-902.
27. Peers S, Montembault A, Ladavière C. Chitosan hydrogels incorporating colloids for sustained drug delivery. Carbohydr Polym. 2022;275:1-36.
28. Mehmood T, Ahmed A. Tween 80 and Soya-Lecithin-Based Food-Grade Nanoemulsions for the Effective Delivery of Vitamin D. Langmuir. 2020;36(11):2886-92.
29. Demisli S, Theochari I, Christodoulou P, Zervou M, Xenakis A, Papadimitriou V. Structure, activity and dynamics of extra virgin olive oil-in-water nanoemulsions loaded with vitamin D3 and calcium citrate. J Mol Liq. 2020;306:112908.
30. Espinoza LC, Silva-Abreu M, Clares B, Rodríguez-Lagunas MJ, Halbaut L, Cañas MA, et al. Formulation strategies to improve nose-to-brain delivery of donepezil. Pharmaceutics. 2019;11(2):1-16.
31. Alkhatib MH, Al-Saedi DA. Cytotoxic Effect of the Combination of Gemcitabine and Atorvastatin Loaded in Nanoparticle on the MCF-7 Breast Cancer Cells and HFS Human Foreskin Cells. Curr Nanosci. 2017;13(6):625-33.
32. Karimi M, Karimian KA, Heli H. A nanoemulsion-based delivery system for imatinib and in vitro anticancer efficacy. Brazilian J Pharm Sci. 2020;56:1-9.
33. Mohafez OM, Alhaider IA, Shehata TM, Mohamed ME. Lupine as a potential agent against diet-induced obesity through adenosine monophosphate-activated protein kinase pathway: Crude oil versus nanoemulsion formulations. Brazilian J Pharm Sci. 2020;56:1-13.
34. Okur NÜ, Onay E, Yaman BK, Sİpahİ H. New topical microemulsions of etofenamate as sufficient management of osteoarthritis. Braz J Pharm Sci. 2022;58:e20123.
35. Sadeq ZA, Mohammed MF. Preparation and in vitro evaluation of topical gel of 5-fluorouracil. J Adv Pharm Educ Res. 2021;11(4):80-5.
36. Songkro S, Isnaini N, Sungkharak S, Tanmanee N, Maneenuan D, Kaewnopparat N et al. Characterization, Antioxidant and Antibacterial Potentials of Tamarindus indica L. Fruit Pulp Extract Loaded O/W Nanoemulsions. Brazilian J Pharm Sci. 2022;58:1-18.
37. Taher SS, Al-Kinani KK, Hammoudi ZM, Ghareeb M Mohammed. Co-surfactant effect of polyethylene glycol 400 on microemulsion using BCS class II model drug. J Adv Pharm Educ Res. 2022;12(1):63-9.
38. Zhu P, Cai L, Liu Q, Feng S, Ruan H, Zhang L, et al. One-pot synthesis of α-Linolenic acid nanoemulsion-templated drug-loaded silica mesocomposites as efficient bactericide against drug-resistant Mycobacterium tuberculosis. Eur J Pharm Sci. 2022;176:106261.