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Self-Emulsifying Drug Delivery System for Enhanced Oral Delivery of Tenofovir: Formulation, Physicochemical Characterization, and Bioavailability Assessment

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Cite This: *ACS Omega* 2024, 9, 8139−8150

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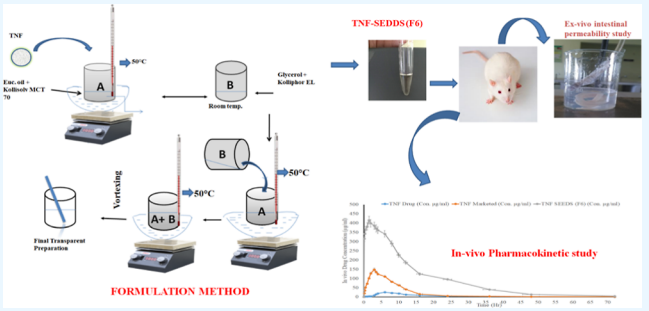
ABSTRACT: Tenofovir (TNF) is a common component of many antiretroviral therapy regimens, but it is associated with poor membrane permeability and low oral bioavailability. To improve its oral bioavailability and membrane permeability, a self-emulsifying drug delivery system (SEDDS) was developed and characterized, and its relative bioavailability was compared to the marketed tablets (Tenof). Based on solubility and ternary phase diagram analysis, eucalyptus oil was selected as an oil phase, Kolliphor EL, and Kollisolv MCT 70 were chosen as surfactant and cosurfactant, respectively, while glycerol was used as cosolvent in surfactant mixture. Optimized SEDDS formulation F6 showed an oil droplet size of 98.82 nm and zeta potential of −13.03 mV, indicating the high stability of oil droplets. Differential scanning calorimetry, X-ray diffraction, and scanning electron microscopy characterization studies were also carried out to assess the amorphous and morphological states of the drug in the prepared SEDDS formulation. The in vitro dissolution profile of SEDDS shows the rapid release of the drug. SEDDS F6 demonstrates a higher drug permeability than the plain TNF and TNF-marketed tablets (Tenof). A pharmacokinetic study in rats revealed that SEDDS F6 showed significantly higher C_{max} and AUC_{0-t} than the marketed tablets and pure drug suspension. In addition, the relative bioavailability of SEDDS formulation dramatically improved by 21.53-fold compared to marketed tablets and 66.27-fold compared to pure drugs. These findings show that SEDDS composed of eucalyptus oil, glycerol, Kolliphor EL, and Kollisolv MCT 70 could be a useful tool for enhancing physiochemical properties and oral TNF absorption. Therefore, SEDDS has shown promise in improving the oral bioavailability of poorly water-soluble drugs.



1. INTRODUCTION

Human immunodeficiency virus (HIV) is a severe ongoing medical illness. Around 40 million individuals are infected with HIV worldwide, which is the main cause of acquired immune deficiency syndrome. The primary target of HIV, a retrovirus belonging to the lentivirus family, is the destruction of CD4+ T cells, an essential component of the immune system needed for normal immune system function. Since 1996, remarkable progress has been made in HIV therapies as a result of the development and continued advancement of highly active antiretroviral therapy (HAART).[1] In HAART regimens, three types of antiretroviral medicines are suggested. Tenofovir (TNF) is a first-line nucleoside reverse transcriptase inhibitor that is widely utilized in treatment.[2] TNF is an adenine analog reverse transcriptase inhibitor with HIV-1 and Hepatitis B antiviral efficacy and approved by the US FDA for the treatment of HIV−AIDS. TNF has been used as a preferred backbone drug in the management of HIV for years and is taken as a 300 mg daily dose; however, it has a low oral bioavailability.[3] TNF has a 2, 17.1, and 5.3% oral bioavailability (BA) in mice, dogs, and monkeys, respectively.[4] Consequently, a delivery method that can manage bioavailability problems using scalable and economical ways is required.

Self-emulsifying drug delivery systems (SEDDS) are an effective way to increase the solubility and bioavailability of poorly soluble compounds. It is an isotropic mixture comprising a drug, a synthetic or natural oil, a cosolvent, a

Received: October 30, 2023
Revised: January 10, 2024
Accepted: January 22, 2024
Published: February 3, 2024

surfactant, and one cosurfactant. Such systems start in the aqueous GIT environment and spontaneously emulsify with the help of GI motility, producing a fine o/w emulsion with a nanometric droplet size of less than 200 nm.[5] In addition to thermodynamic stability, SEDDS may be compared to submicrometer and metastable emulsions as a more effective, practical, and patient-friendly method since SEDDS can be immediately packed into soft and hard gelatin capsules for practical oral delivery as a unit dose form. Additionally, following the solubilization of SEDDS in the in situ lymphatic route, the drug can be absorbed, bypassing hepatic first-pass effect loading and resulting in increased bioavailability.[6] Furthermore, SEDDS has been highlighted as an essential method for drug delivery because of the small size of the droplet and high solubilization potential, which could improve permeability across the GI membrane.[7] To choose an acceptable self-emulsifying formulation, factors such as a drug's solubility in different components, the self-emulsifying band in the phase diagram, and the droplet size distribution of the resulting emulsion are taken into consideration.[12] In addition, SEDDS can be produced more easily and inexpensively than other nanocarriers like liposomes and nanoparticles, which is a considerable advantage.[8]

An extensive examination of the literature reveals that SEDDS have not been used to increase the bioavailability of TNF. Therefore, the current work aimed to develop SEDDS of TNF to enhance TNF oral bioavailability. To achieve this, the solubility of TNF in several excipients was assessed, and the oil, surfactant, and cosurfactant that had the highest solubility for TNF were chosen for formulating TNF-SEDDS. The selected formulation of SEDDS was characterized in terms of droplet size, zeta potential, Fourier transform infrared spectroscopy, differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), and in vitro dissolution. Finally, ex vivo permeability and pharmacokinetic studies in rats were also carried out to evaluate the improvement in the oral absorption of TNF-SEDDS.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. Emcure laboratories. Pune, India, provided TNF as a free sample. Kolliphor EL (polyoxyl castor oil), Kollisolv MCT 70 (medium chain triglycerides), and Kolliphor HS15 (polyoxyl 15 hydroxystearate) were complementary samples provided by BASF, Mumbai, India. Raj Chemicals provided glycerol. Propylene glycol and caprylic acid were purchased from Loba Chemicals, Mumbai, India. The analytical grade was used for all other chemicals.

2.2. Solubility Studies. The solubility of the TNF was tested in different types of oils surfactants and cosurfactants with the shake flask technique. An excess TNF was mixed into a screw-capped vial holding 2 mL of vehicle. The mixture was shaken on a mechanical shaker (Remi, Mumbai, India) for 48 h before settling for 24 h. The excess insoluble TNF was removed after centrifuging the equilibrated liquid at 5000 rpm for 10 min. The amount of TNF in the filtrate was determined by appropriate dilution using a UV−vis spectrophotometer (UV-1800, Shimadzu, Japan) at 260 nm.[9]

2.3. Construction of Pseudoternary Phase Diagram. The aqueous titration method was used to generate a pseudoternary phase diagram to determine the concentration range of components for the SEDDS. First, the surfactant−cosurfactants mixture (S_{mix}) was prepared by mixing surfactant (Kolliphor EL) into glycerol solution (1:1, w/w) and cosurfactant (Kollisolv MCT 70) according to a certain mass ratio (K_m = 3:1, 2:1, 1:1, 1:2, 4:1). Pseudoternary phase diagram was prepared by titrating a homogeneous combination of oil, S_{mix}, and water.[10] The oil (eucalyptus oil) and S_{mix} were mixed at various ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 in numerous vials. The oil and S_{mix} were titrated with distilled water. Each time water was added, the mixture was vortexed to homogenize it before the sample was examined for differences in optical clarity. At equilibrium, the mixtures were examined for a key change in transparency. Pseudoternary phase diagrams were created for each S_{mix} to find a suitable S_{mix} ratio.

2.4. Formulation of SEDDS. Following the identification of the monophasic region, different formulation batches were prepared. For the preparation of SEDDS, S_{mix} (3:1) was selected. In an isothermal water bath at 50 °C, 8 mg of TNF was accurately weighed and dissolved in 1.2 g of Kollisolv MCT 70 with 1.08 g of eucalyptus oil in beaker-A. The drug dissolves in a mixture of cosurfactant and oil, in beaker-A, within 1−2 min. After that, 1.8 g of glycerol and 1.8 g of Kolliphor EL were mixed for 2 min at room temperature in a different beaker-B. The mixture (beaker B) was then gradually put dropwise into the beaker-A mixture. Then, this combined mixture was vortexed until a clear preparation was obtained. S_{mix} (3:1) was diluted in oil at several concentrations (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) before being titrated with water. A vortex was used to uniformize the mixture, and the turbidity was visually assessed.[11]

2.5. Thermodynamic Stability Studies. The stability of colloidal drug delivery systems during storage is a critical hurdle to overcome before such formulations can be made available in clinical settings. Therefore, different thermodynamic stability studies were performed on several selected formulations. The formulations were centrifuged in a centrifugation machine (REMI, India) for 30 min at 5000 rpm. The formulations that did not show any instability, including phase separation, cracking, or creaming, were subjected to further heating−cooling cycle tests. This test uses six temperature cycles between 4 and 40 °C, with storage at a temperature lasting at least 48 h. These formulations were further tested for phase separation, creaming, as well as cracking.

2.6. Self-Emulsification Evaluation. The USP dissolution apparatus type II was used to evaluate the self-emulsification effectiveness of SEDDS.[12] 0.5 mL portion of each formulation (F1−F9) was added dropwise to 500 mL of distilled water at 37 ± 0.5 °C. A conventional dissolving paddle made of stainless steel was rotated at 50 rpm to provide gentle agitation. Emulsification time was observed visually. Grade A formulation (which forms microemulsions quickly within one min that are transparent or bluish in appearance) that passed the rapid physical stability and self-emulsification tests was selected for further study.

2.7. Preparation of SEDDS Powder for Solid-State Characterization. Although there are several techniques for solidifying liquid SEDDS, adsorption over carriers is the most widely utilized and industrially applied technique.[13] Different carriers, including Neusilin US2, mannitol, and calcium silicate, were used to adsorb the TNF-SEDDS liquid formulation. Initially, 300 mg of each of the adsorbents listed above was combined with a set weight of TNF-SEDDS (100 mg) in a

https://doi.org/10.1021/acsomega.3c08565
ACS Omega 2024, 9, 8139−8150

mortar, and it was evenly mixed to create a bulk mass. Then, the prepared mass was passed through sieve no. 80 to get a consistent particle size. The final SEDDS was kept in desiccators until further analysis. After the physical observation and flow properties, Neusilin US2 was found to be better as compared with calcium silicate and mannitol. Therefore, TNF-SEDDS-Neusilin was selected for solid-state characterization studies.

2.8. Characterization of SEDDS Formulations. *2.8.1. Globule Size Analysis and Zeta Potential Measurement.* The polydispersity index (PDI) and droplet size distribution of formulation were evaluated using photon correlation spectroscopy, which analyzes changes in light scattering caused by the Brownian motion of particles.[14] Appropriate dilution (0.1 mL of the formulation was diluted to 100 mL) of the sample was done, and the sample cell was then filled with an aliquot to measure the droplet size. Using a cumulative analysis of the results from the three replications, the average droplet size and PDI were determined using a Zetasizer Nano-ZS90 (Malvern Instruments, Malvern, UK). On the same apparatus, a second electrode was used to measure the zeta potential values of the same samples.

2.8.2. Fourier Transforms Infrared Spectroscopy Analysis. Fourier transforms infrared (FTIR) spectroscopy is used to assess the compatibility of the excipients such as Kolliphor EL, Kollisolv MCT 70, eucalyptus oil, and glycerol with TNF. It also helps in determining the compatibility of excipients in the formulation of SEDDS. Neusilin US2 was used as a solidifying agent to cover SEDDS into powder form for FTIR analysis. The FTIR spectrum was examined between 4000 and 500 cm^{-1} scanning range using a Shimadzu FTIR spectrometer (IR Affinity 1Model, Japan).[15]

2.8.3. Differential Scanning Calorimetry Analysis. The thermal behavior of drugs and interactions between drugs and excipients were evaluated with a DSC (DSC-PYRIS-1, PerkinElmer, USA).[16] A powdered TNF-SEDDS (F6) formulation was prepared using Neusilin US2 for DSC analysis. In a dry nitrogen environment, tests were conducted. Samples were weighed and crimped into aluminum pans and heated at 0 to 200 °C at a rate of 10 °C/min under an inert atmosphere. The reference cell was an empty, crimped aluminum pan.

2.8.4. Powdered X-ray Diffractometry Analysis. The powdered TNF-SEDDS (F6) formulation was used for PXRD analysis. Neusilin US2 was used as a solidifying agent to cover SEDDS into powder form for XRD analysis. An XRD (Bruker D8 Advance, WI, USA) with a scanning rate of 2°/min was used to analyze the physical states of TNF-SEDDS powder over a range of 5−60 (θ).[17]

2.8.5. Scanning Electron Microscopy Analysis. The surface characteristics of powdered TNF-SEDDS (F6) formulation, Neusilin, and TNF were characterized by SEM (JEOL JSM 5400). Carbon tape with a double-sided adhesive was used on the aluminum stubs. The sample of powder was dispersed all over the tape. Using a JFC-1600 auto fine coater, a platinum plasma beam was applied to the aluminum stubs for 25 min to generate a 2 nm-thick coating on top of the dispersed powder. These stubs were then inserted into a SEM vacuum chamber. To determine the samples' shape, they were examined using an XL 30 gaseous secondary electron detector (acceleration voltage: 30 kV, working pressure: 0.8 Torr).

2.9. In Vitro Drug Release Study. The dissolution test apparatus USP type II was utilized to perform the in vitro dissolution evaluation of the SEDDS formulation using the dialysis bag technique. The liquid and solid TNF-SEDDS formulation F6 was inserted into the dialysis bag, locked with a clamp, and placed in 900 mL of phosphate buffer pH 6.8 dissolution media at 37 °C. The paddle's rotational speed was kept at 50 rpm. At predetermined time intervals of 5, 15, 30, 45, and 60 min, 5 mL aliquots were taken out, and the same volume of fresh dissolution medium was replenished to maintain the sink conditions. The aliquots were subjected to UV spectroscopy at 260 nm for analysis. The release of the drug from liquid and solid SEDDS formulations was compared with those of the pure drug suspension and marketed diffused tablets (Tenof).

2.10. Ex Vivo Intestinal Permeability. The noninverted rat intestinal sac method was used for the ex vivo intestinal permeability study.[18] The gut permeation study protocol was approved by the Institutional Animal Ethics Committee of Dadasaheb Balpande College of Pharmacy (DBCOP/IEAC/052020-09). Male Wistar rats (weight 200−250 g) had fasted for 12 h before euthanasia with free access to water and were housed under standard conditions. The animal was slaughtered, and the small intestine's ileum portion was removed and sliced longitudinally. The Ileum part of 5 cm was cut and washed with pH 6.8 phosphate buffer before being filled with 2 mL of formulation and knotted with thread at both ends. This was put in a 30 mL beaker of pH 6.8 phosphate buffer and stirred with a magnetic stirrer. Samples were withdrawn at predetermined intervals and analyzed with UV spectroscopy at 260 nm. Permeation study of drugs from the SEDDS formulation was compared with the pure drug suspension and marketed diffused tablets (Tenof).

2.11. In Vivo Pharmacokinetic Studies. *2.11.1. Study Protocol.* The oral bioavailability study of SEDDS formulation was investigated according to the approved protocol by the Institutional Animal Ethics Committee of Dadasaheb Balpande College of Pharmacy (DBCOP/IEAC/052020-09). Male Wistar rats (weight 200−250 g) were used to calculate the oral bioavailability of the TNF-SEDDS formulation F6, Tenof dispersion, and the TNF pure drug suspension at a dosage of 10 mg/kg of body weight. Animals were divided into four groups, and each group had six animals that were starved for 8 h while having free access to water. Animals were kept in a controlled environment with a 22 ± 2 °C temperature and 50 ± 10% humidity. Standard laboratory meals and water were available on a needy basis. Group I served as control, Group II was given SEDDS formulation F6, Group III was given Tenof suspension, and Group IV received pure drug suspension. Blood samples were taken at 0 (predosage), 0.5, 1, 2, 3, 4, 6, 8, and 24 h in a microcentrifuge tube containing ethylenediaminetetraacetic acid. The blood samples were centrifuged at 3000 rpm for 30 min. The plasma was separated and kept in the refrigerator at −20 °C until analysis, which was carried out using the RP-HPLC method. The sample must be at room temperature for bioanalysis, and the drug was separated using a protein precipitation procedure with acetonitrile.

2.11.2. Sample Preparation and Bioanalytical Method. TNF was quantitatively determined in plasma by RP-HPLC using a mobile phase consisting of 10 mM potassium dihydrogen phosphate buffer (pH 6.8), and acetonitrile in the ratio (65:35) at a flow speed of 1 mL min^{-1}.[19] 50 μL of the IS solution (Afatinib Dimaleate) and 50 μL of the drug solution (100 μg/mL) were combined with 100 μL of plasma.

https://doi.org/10.1021/acsomega.3c08565
ACS Omega 2024, 9, 8139−8150

500 μL of the acetonitrile (protein precipitator) was added, and the mixture was then vortexed for 1 min. Then, the solution was centrifuged at 10,000 rpm for 20 min in a centrifuge. The supernatant was filtered, and 20 μL of the filtrate was added to the high-performance liquid chromatography (HPLC) system (RF6000, Shimadzu, Japan). With a retention time of 4.810 min for IS and 8.339 min for TNF at wavelength 254 nm, the system was shown to yield peaks for TNF that are sharp and well resolved. The calibration plot was made across the concentration range of 0.05−10.0 μg/mL, and the TNF content in samples was quantified using this plot.

2.11.3. Pharmacokinetic Parameters. Noncompartmental analysis, also referred to as Model independent analysis, was used to calculate the pharmacokinetic parameters.[20] All pharmacokinetic parameters (T_{max}, C_{max}, AUC_{0-t}, and $T_{1/2}$) were calculated individually for each subject in the group, and the values are expressed as mean ± SD. The comparative in vivo bioavailability profiles of developed SEDDS formulation F6, Tenof tablet suspension, and the pure drug suspension were also determined.

2.11.4. Statistical Analysis. The GraphPad Prism tool (InStat 3.06, Software Inc., San Diego, CA, USA) was used to statistically analyze the pharmacokinetic results, and the Student's *t*-test was used to determine significant differences.

3. RESULTS AND DISCUSSION

3.1. Screening of Oil, Surfactant, and Cosurfactant. The best excipients can be preliminarily selected through a saturation solubility experiment. The solubility profiles of TNF in various mediums are shown in Table 1. Eucalyptus oil was selected as the oil phase as it showed the highest solubility of TNF (246 ± 2.48 μg/mL). More drug solubility in the oil phase is needed to maintain the drug's solubilized condition in an o/w microemulsion because the role of surfactant or cosurfactant to drug solubility is decreased after dilution in GIT. Glycerol and Kolliphor EL had the maximum TNF solubility among the cosolvent and surfactants tested. The proper high and low HLB surfactant ratios cause the development of a stable microemulsion when diluted with water. A single surfactant seldom provides the fluid interfacial film, and short negative interfacial tension is needed to increase microemulsions' stability. Cosurfactants normally decline to interface bending stress and provide the interfacial layer sufficient elasticity/flexibility to take up the varying curves needed to form microemulsion throughout a broad range of compositions. Kollisolv MCT 70 is being investigated as a cosurfactant since it dissolves more readily than TNF. A pseudoternary phase diagram was used to further investigate the optimal excipients.

Table 1. Solubility of Tenofovir in Different Oils, Surfactants, and Co-Surfactants

vehicles	solubility (μg/mL)
propylene glycol	202.63 ± 1.25
glycerol	2879 ± 2.52
coconut oil	74.2 ± 0.23
cremophor RH 40	133.4 ± 1.36
eucalyptus oil	246 ± 2.48
olive oil	18.51 ± 0.15
Brij 35	144.9 ± 1.82
caprylic acid	167.55 ± 2.17
Kolliphor EL	310.185 ± 3.65
Kollisolv MCT 70	344.39 ± 3.87
Kolliphor HS 50	178.2 ± 1.42

3.2. Pseudoternary Phase Diagram Studies. It is used to determine the ideal component concentrations for the self-emulsifying region and to produce the SEDDS formulation. Utilizing a binary mixture of surfactants improves the hydrophilic−lipophilic balance of surfactants. This enhances the surfactant layer's flexibility at the o/w interface as well as its localization there, both of which contribute to stabilizing the o/w microemulsion.[21] The pseudo−ternary phase diagrams were constructed for different S_{mix} ratios, namely, S_{mix} 3:1, 2:1, 1:1, 1:2, and 4:1. S_{mix} represents the mixture of Surfactant and cosurfactant in different ratios, as mentioned above. The surfactant Kolliphor EL was mixed with glycerol in a 1:1 ratio to the desired HLB value to form an o/w microemulsion. The cosurfactant Kollisolv MCT 70 was added here to make the film of microemulsion more flexible and not allow the separating oil and water phase.[22]

Figure 1 displays the pseudoternary phase diagram for the S_{mix} (A = 3:1, B = 2:1, C = 1:1, D = 1:2, and E = 4:1). The largest microemulsion zone is seen for 3:1 vs 2:1 or 1:1 when comparing the phase diagrams reported for S_{mix} ratios of 1:2. Additionally, the 2:1 S_{mix} ratio exhibits a greater microemulsion zone than the 1:1 ratio. These findings suggested that a higher surfactant favors a greater microemulsion area. When the microemulsion region in the 3:1 S_{mix} ratio was studied and compared with a further higher ratio of surfactant versus cosurfactant i.e. 4:1 dramatically microemulsion region was decreased. According to the outcomes, a S_{mix} ratio of 3:1 is ideal for the formulation of microemulsion. Different formulation batches (F1−F9) were selected from the pseudo ternary phase diagram of S_{mix} 3:1, having varied oil, S_{mix}, and water percentages (Table 2). The low oil percentage ratio formulation has greater stability and smaller oil droplet size in o/w microemulsion.[23] The oil ratios were selected in such a way that the dose of a drug must be easily soluble in oil. All the selected formulations were subjected to thermodynamic stability studies to ensure that the developed formulations were stable when subjected to centrifugation study, freeze and thaw cycle. After 30 min of centrifugation at 5000 rpm, the F6 SEDDS formulation did not show any phase separation, creaming, or cracking. During the freeze and thaw cycle, phase separation was also not observed with the F6 formulation. No creaming or cracking after six cycles at 4 and 40 °C during 48 h of storage at each temperature was seen with the F6 formulation. Another important fact about the F6 formulation is that it contains the minimum percentage of surfactant among all formulations. It was also reported by researchers that lower surfactant concentration minimum irritation scores were achieved hence a higher safety profile.[24]

Oral SEDDS eventually undergo significant dilution in GI fluids to generate orally water-soluble microemulsions. The self-emulsification test is used to examine whether the formulation creates a microemulsion quickly when mixed with double distilled water.[25] Formulation F6 passed the test by promptly generating (within 1 min) a clear microemulsion.

3.3. Characterization of Optimized SEDDS Formulations. *3.3.1. Globule Size and Zeta Potential Measurement.* The size of the emulsion globules in a SEDDS formulation primarily determines the drug release behavior and absorption. Therefore, while evaluating the efficacy of SEDDS for drug delivery, the globule size analysis is a crucial factor. Globule

https://doi.org/10.1021/acsomega.3c08565
ACS Omega 2024, 9, 8139−8150

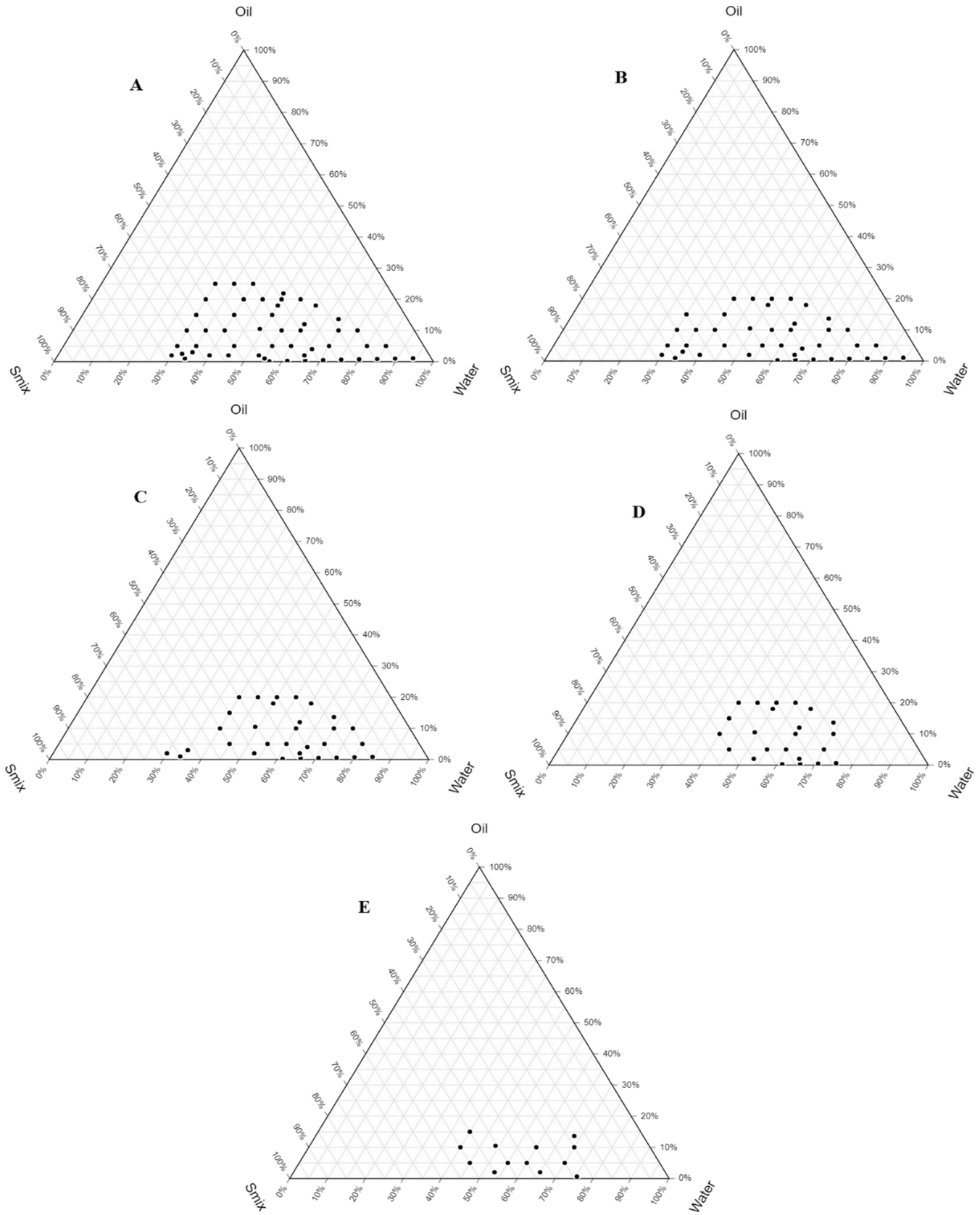


Figure 1. Pseudoternary phase diagram for S_{mix} (A = 3:1, B = 2:1, C = 1:1, D = 1:2 and E = 4:1).

size analysis of formulation F6 was found 98.82 nm (Z-average), as shown in Figure 2A. The interfacial film is most likely made larger by the presence of cosurfactants, which explains the mean globule size increases as the cosurfactants concentration rises. An increase in electrostatic repulsive forces often prevents the formation of coalescence between micro-

https://doi.org/10.1021/acsomega.3c08565
ACS Omega 2024, 9, 8139−8150

Table 2. Formulation Batches Developed for Ratio 3:1 with Different Concentrations for Optimization

S. no	ingredients	F1 (1:9)	F2 (2:8)	F3 (3:7)	F4 (4:6)	F5 (5:5)	F6 (9:1)	F7 (8:2)	F8 (7:3)	F9 (6:4)
1	tenofovir	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg	8 mg
2	eucalyptus oil	0.12 g	0.24 g	0.36 g	0.48 g	0.6 g	1.08 g	0.96 g	0.84 g	0.72 g
3	glycerol	16.2 g	14.4 g	12.6 g	10.8 g	9 g	1.8 g	3.6 g	5.4 g	7.2 g
4	Kolliphor EL	16.2 g	14.4 g	12.6 g	10.8 g	9 g	1.8 g	3.6 g	5.4 g	7.2 g
5	Kollisolv MCT 70	10.8 g	9.6 g	8.4 g	7.2 g	6 g	1.2 g	2.4 g	3.6 g	4.8 g

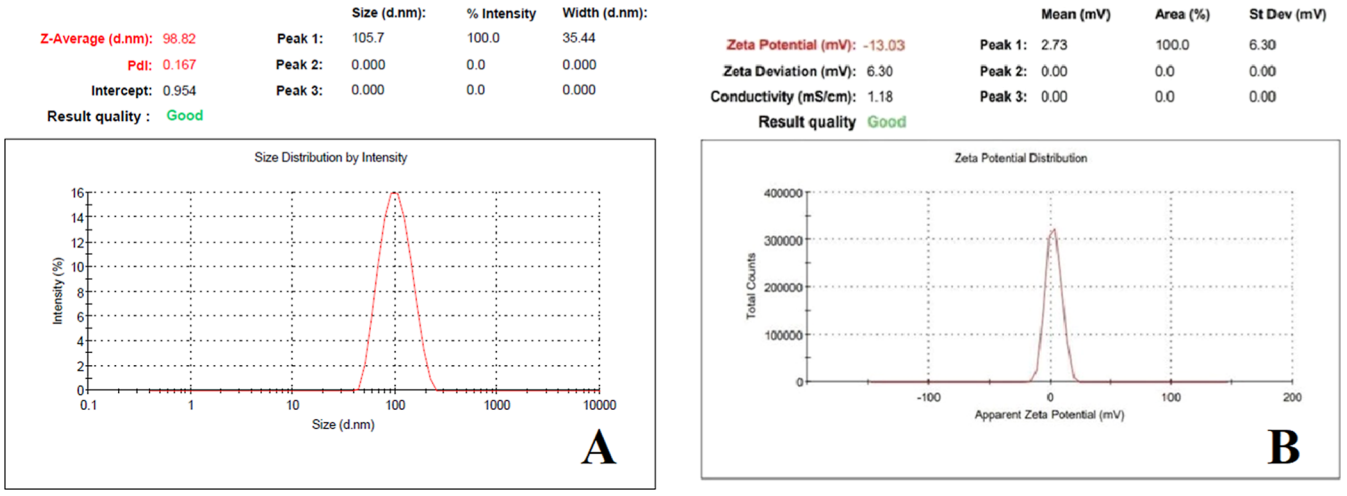


Figure 2. Globule size (A) and zeta potential (B) for the TNF-SEDDS F6 formulation.

emulsion droplets. Phase separation will occur if electrostatic repulsive forces decrease.[26] Zeta potential measurements were performed on SEDDS F6 and were found to be −13.03 mV, shown in Figure 2B which is negatively charged because of the free fatty acid.

3.3.2. FTIR Spectroscopic Analysis. The primary FTIR peaks of pure TNF, glycerol, Kolliphor EL, Kollisolv MCT 70, eucalyptus oil, formulation F6, and the physical mixture were determined. The drug's standard absorption bands and linkages between various functional groups were not altered significantly. There was no well-defined interaction found between the drug and the excipients used, as shown by the FTIR spectrum in Figure 3A−G.

3.3.3. DSC Analysis. The drug's thermotropic behavior and physical state in SEDDS were examined using DSC analysis (Figure 4). Figure 4A−C depicts the DSC thermograms of TNF, Kolliphor EL, and Kolliphor MCT 70 whereas Figure 4D depicts the DSC thermograms of fresh SEDDS (F6) with Nesulin. The peak of the phase transition for pure drug TNF at 132.71 °C with significant enthalpy supported the crystalline form of TNF (Figure 4A). When Kolliphor EL and Kolliphor MCT 70 were utilized as carriers in SEDDS, they showed a large endothermic peak, indicating their amorphous nature (Figure 4D). After three months (3 M) of stability, there was no identifiable TNF peak in the SEDDS (F6) formulation, indicating the stable physical state of the drug (Figure 4E).

3.3.4. Spectroscopic Analysis Using PXRD. The TNF drug's PXRD spectrum and SEDDS (F6) XRD pattern overlay are shown in Figure 5. The high-intensity diffraction peaks of the pure drug at 7.18° of 9.58, 10.38, 11.10, 12.18, 13.89, 21.98, 22.53, 24.25, and 31.54° demonstrated the crystal structure of the material (Figure 5A). Figure 5B,C shows diffuse peaks for Kolliphor EL and Kolliphor MCT 70, suggesting an amorphous condition, while Figure 5D,E demonstrates fresh and stable after three months without any discernible peaks for TNF.

3.3.5. Morphological Analysis Using SEM. The SEM images of the surface morphology of TNF and SEDDS (F6) formulations are shown in Figure 6. Figure 6A shows the crystalline nature of TNF whereas Figure 6B shows the SEM image of TNF-SEDDS F6 formulation. The SEM image of TNF-SEDDS F6 showed an amorphous transition (Figure 6B).

3.4. In Vitro Drug Dissolution Study. Figure 7 depicts the drug release profile of the optimized TNF-SEDDS F6 formulation (solid-S, liquid-L) in comparison to pure TNF and marketed TNF-diffused tablets (Tenof). The formulation liquid TNF-SEDDS (F6)-L was thought to have the fastest and complete drug release. The drug release from F6 reached more than 50% within 30 min and then attained a value of around 80% at 45 min and reached more than 90% by a time of 60 min. However, the drug release from liquid SEDDS (L-SEEDS) is slightly higher than the solid SEDDS (S-SEDDS) formulation. The S-SEDDs have slightly delayed release compared to L-SEDDs because S-SEDDs needed more steps such as desorption of adsorbed SEDDs from the Neusilin US2 during the dissolution process.[27] Formulation F6 SEDDs has the fastest release due to its small globule size and low PDI. TNF's solubility was significantly improved by the selected oil, surfactant, and cosurfactant. Hence, SEDDS F6 is expected to quickly dissolve TNF in the GI fluid after taking it, making it easier to absorb via oral delivery.

3.5. Ex Vivo Intestinal Permeability. Figure 8 depicts intestinal drug permeability of the TNF-SEDDS F6 formulation, TNF, and TNF-marketed preparation Tenof. TNF-SEDDS F6 has 93.12% permeation within 5 h through rat intestinal tissue, whereas TNF pure drug and TNF-marketed preparation Tenof have 48.61 and 72.56% respectively. Incorporating TNF into SEDDS is an important factor for improving the penetration rate because it improves absorption

https://doi.org/10.1021/acsomega.3c08565
ACS Omega 2024, 9, 8139−8150

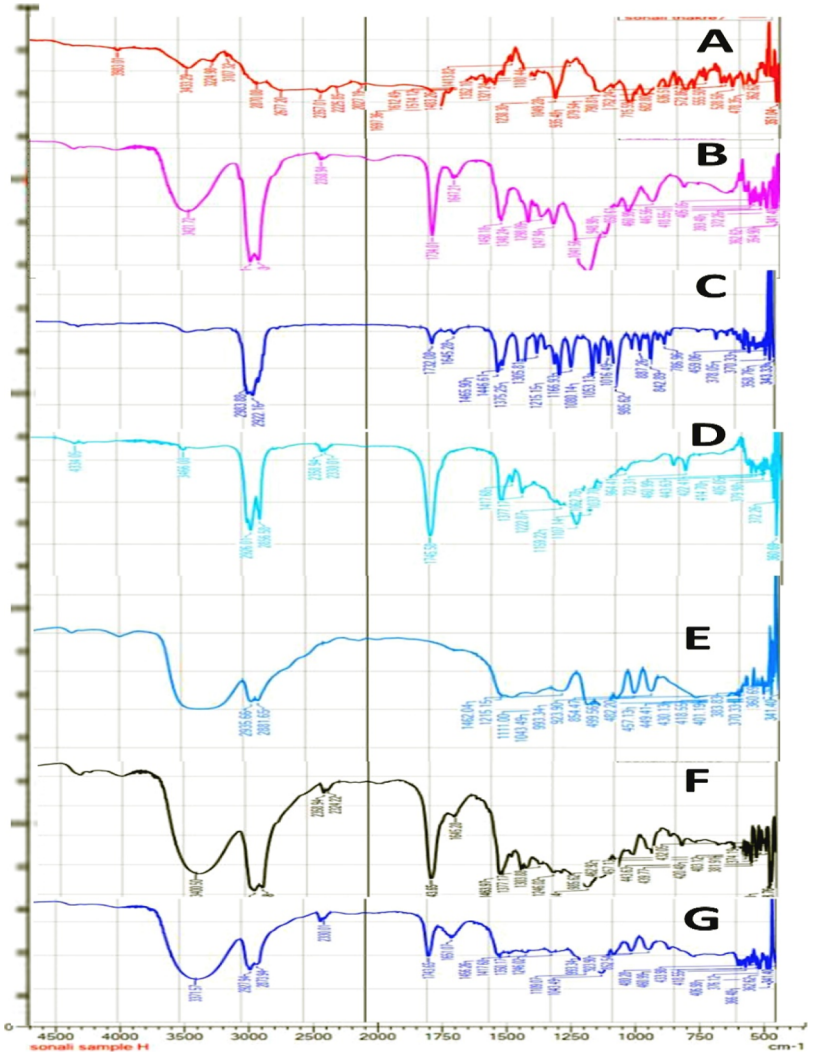


Figure 3. FT-IR spectra of pure tenofovir (A), eucalyptus oil (B), Kolliphor EL (C), Kollisolve MCT 70 (D), glycerol (E), physical mixture (F), and TNF-SEDDS F6 (G).

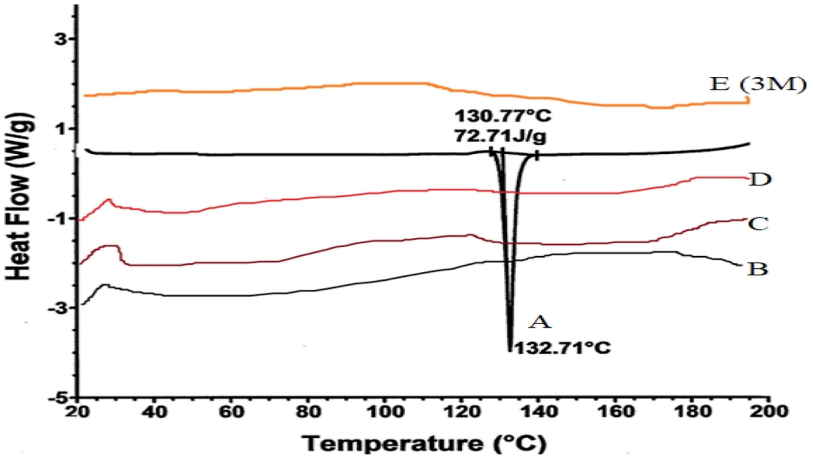


Figure 4. DSC spectra of tenofovir drug (A), Kolliphor EL (B), Kollisolv MCT 70 (C), TNF-SEDDS F6 (D), and TNF-SEDDS F6- 3 M (after 3 months) (E).

by keeping the drug solubilized at absorption sites. Additionally, the nanosized droplets of SEDDS offer more interfacial surface area for drug release and a faster penetration process.[28,29] Therefore, the TNF-SEDDS F6 formulation has been shown to have much more drug penetration than the commercial tablet and API.

3.6. In Vivo Pharmacokinetic Studies. Pharmacokinetic studies in Wistar rats were performed to evaluate the

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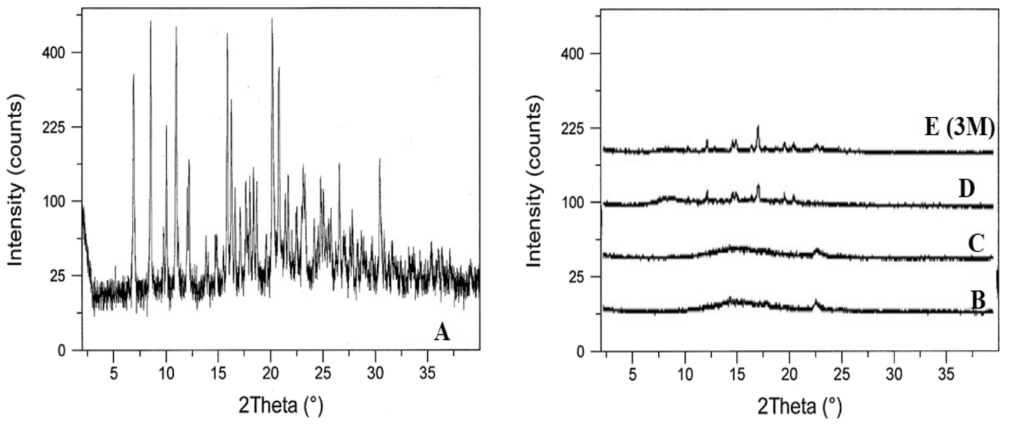

Figure 5. XRD spectra of tenofovir drug (A), Kolliphor EL (B), Kollisolv MCT 70 (C), TNF-SEDDS F6 (D), TNF-SEDDS F6-3 M (after 3 months) (E).

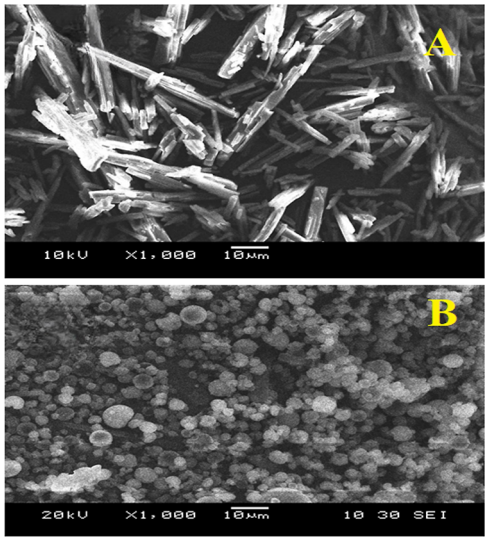

Figure 6. SEM images of tenofovir drug (A), TNF-SEDDS F6 (B).

effectiveness of SEDDS in improving TNF bioavailability. HPLC quantification of TNF in plasma samples was done as per the validated method. Figure 9A represents the internal standard chromatogram of Afatinib Dimaleate (RT = 4.810 min) and drug tenofovir (RT = 8.339 min) at wavelength 254 nm. The calibration curves' linear regression analysis revealed a strong linear association across the concentration range of 0.05−10 μg mL^{-1}, with R^2 = 0.9996 (Figure 9B). Rat pharmacokinetics was successfully measured using this bioanalytical method.

Figure 10 shows the plasma drug concentration versus time profile after a single dosage administration of the TNF-SEDDS formulation (F6), TNF dispersed tablet (Tenof), and TNF pure drug suspension. Kinetica version 5.0 (Thermo Fischer Scientific, Waltham, MA, USA) software was used to compute pharmacokinetic parameters using noncompartmental analysis. The pharmacokinetic parameters (T_{max}, AUC_{0-t}, $T_{1/2}$, and C_{max}) are shown in Table 3. The data were statistically compared using the GraphPad Prism software and the student-independent samples test with a p-value $\leq$ 0.0001. The C_{max} value for the SEDDS (F6) formulation (415.26 ± 2.11 μg/mL) was significantly increased compared to TNF tablet (Tenof) suspension (148.57 ± 2.54 μg/mL), and TNF pure drug suspension (25.69 ± 2.14 μg/mL). On the other hand, the time to achieve the maximal concentration (T_{max}) of the F6 formulation (1.55 ± 1.18 h) was less than that of the TNF tablet (Tenof) suspension (3.02 ± 1.42 h) and TNF pure drug suspension (6.15 ± 1.15 h). The mean AUC_{0-72} for the SEDDS F6 formulation was calculated to be 11546.64 ±

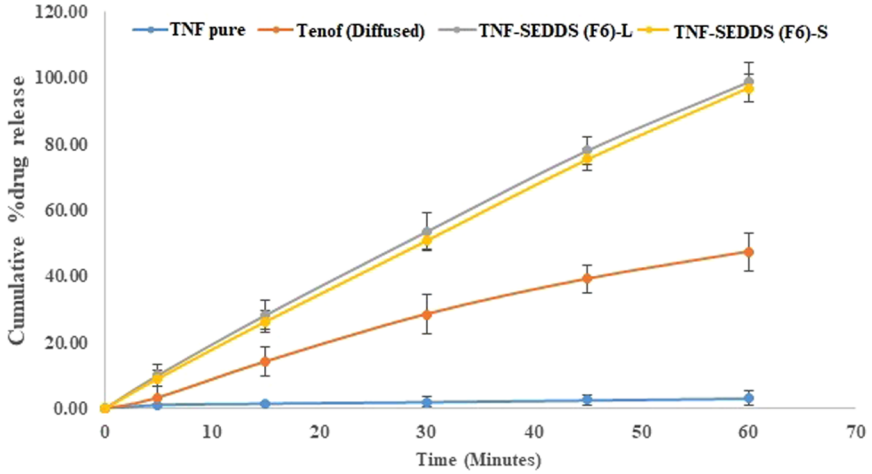

Figure 7. Drug release profile of the optimized formulation TNF-SEDDS (F6)-L and TNF-SEDDS (F6)-S in comparison to the drug TNF and marketed TNF-diffused tablets (Tenof).

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ACS Omega 2024, 9, 8139−8150

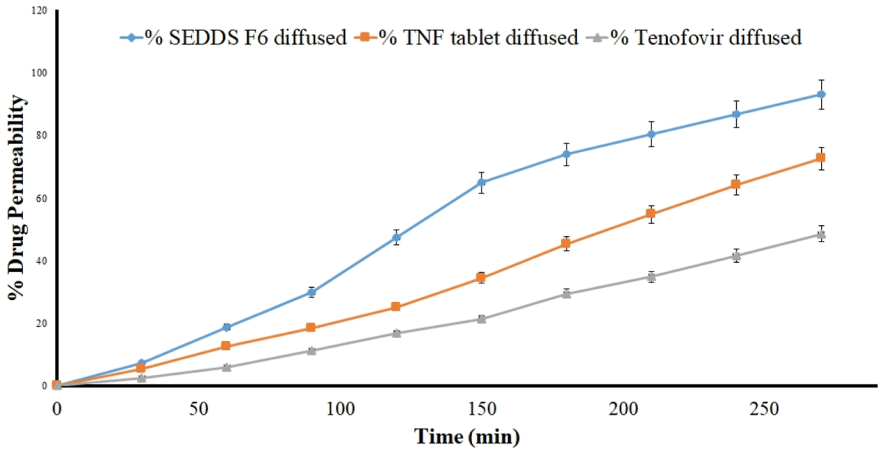

Figure 8. Ex vivo intestinal stomach permeability for drug TNF, TNF-diffused tablet (Tenof), and TNF-SEDDS (F6) formulation.

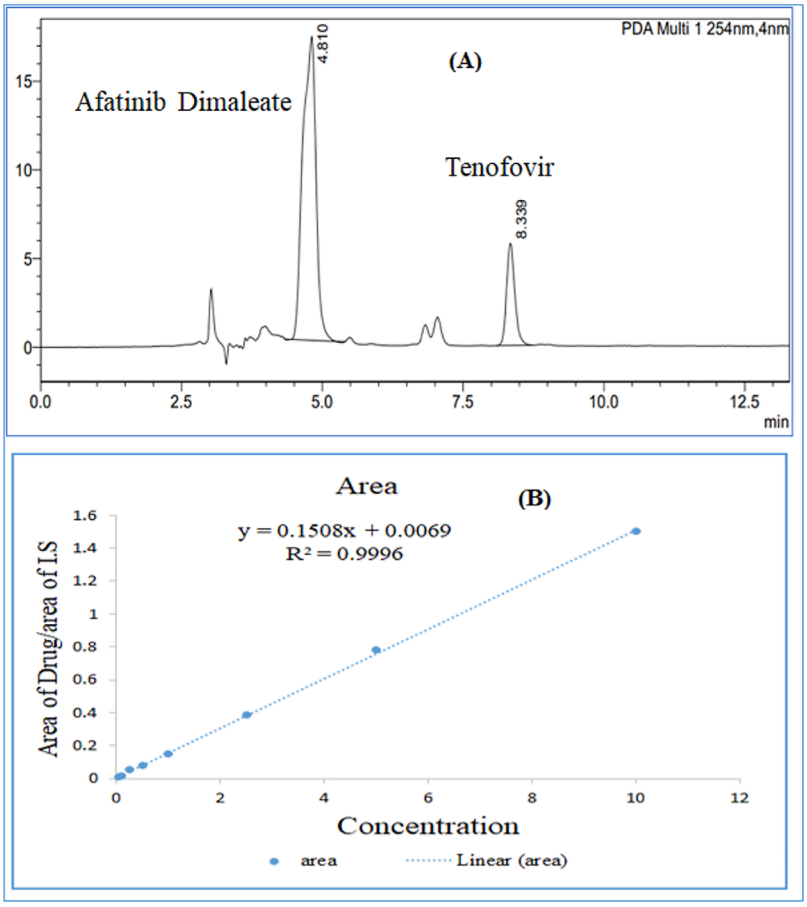

Figure 9. HPLC chromatogram of internal standard (Afatinib Dimaleate) and tenofovir. (A) Calibration plot (B).

139.82, which showed a 21.52-fold increase in the bioavailability of the drug in comparison to the TNF-marketed tablet (Tenof) and 66.27-fold increase in the bioavailability of the drug compared with the API suspension of the drug.

The higher value of C_{max} and AUC of SEDDS ensures that higher availability of the drug at the site of action and shorter T_{max} value could indicate the fast onset of action as compared to pure drugs and marketed formulation (Tenof tablets).[30] The reason behind the increased bioavailability of TNF from SEDDS may be due to the following factors: (a) increased surface area brought about by the microemulsion droplets, (b) increased diffusion of fine droplet emulsion, and (c) increased mucosal permeability of drug due to surfactant in the SEDDS.[31] Surfactants not only increase the drug's dissolution rate but also make the drug more permeable by rupturing the lipid bilayer of the intestinal membrane.[6] These data demonstrate that incorporating the drug into a SEDDS could increase the oral bioavailability of TNF.

4. CONCLUSIONS

Tenofovir was successfully transformed into a SEDDS in the current study using glycerol, Kolliphor EL, and Kollisolv MCT

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ACS Omega 2024, 9, 8139−8150

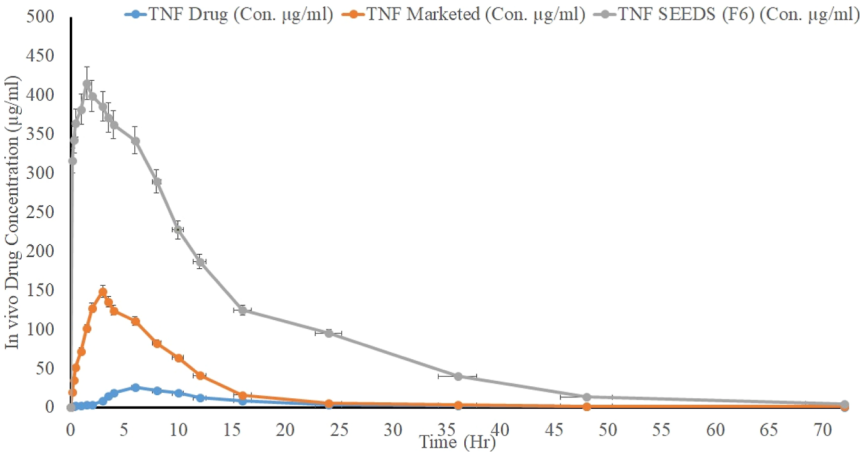

Figure 10. Plasma drug concentration vs time profile following single-dose administration of TNF drug, TNF diffused tablet (Tenof), and TNF-SEDDS (F6) formulation.

Table 3. In Vivo Pharmacokinetic Parameters for the TNF Drug, TNF-Diffused Tablet (Tenof), and TNF-SEDDS (F6) Formulation

parameters	TNF drug	TNF marketed (Tenof)	TNF-SEDDS (F3)
C_{max} (μg/mL)	25.69 ± 2.14	148.57 ± 2.54	415.26 ± 2.11
T_{max} (h)	6.15 ± 1.15	3.02 ± 1.42	1.55 ± 1.18
AUC_{0-72} (μg h/ml)	174.23 ± 58.18	536.41 ± 131.54	11546.64 ± 139.82
$t_{1/2}$ (h)	17.25 ± 1.24	17.28 ± 1.51	17.21 ± 1.33

70 as surfactants and cosurfactants. To prepare SEDDS, a 3:1 (S_{mix}: cosurfactant) ratio was employed. The optimized emulsion's droplet size was also measured and found to be in the nano range. To rule out interaction, FTIR analysis revealed the retention of significant TNF peaks in the formulation. The amorphization of crystalline TNF in the SEDDS formulation system was discovered using DSC and XRD studies. According to SEM examination, the drug changed from its crystallized state to an amorphous state with a decreased morphological pattern compared to that of its spherical carrier. The TNF-SEDDS F6 in vitro dissolution profile showed a rapid drug release compared to its marketed formulation and API. The relative ex vivo diffusion study of TNF-SEDDS formulation demonstrates higher drug permeability than the plain drug TNF and TNF-marketed tablet (Tenof). Pharmacokinetic studies in Wistar rats revealed that prepared TNF-SEDDS (F6) had better oral bioavailability than the TNF-marketed formulation (Tenof) and pure TNF. Thus, our study provides a useful oral dosage form for tenofovir.

■ ASSOCIATED CONTENT

Data Availability Statement

Data is contained within the article.

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N.M.; R.F. and S.T.—conceptualization, supervision, investigation, methodology, writing—original draft; M.A.M. and M.S.A.—formal analysis, methodology, data curation writing—original draft; M.S.A. and S.S.A.—methodology, Data curation, writing—review and editing; M.K.A. and M.F.A. resources, software, validation, writing—review and editing; D.M.—resources, writing—review and editing. All authors contributed

https://doi.org/10.1021/acsomega.3c08565
ACS Omega 2024, 9, 8139−8150

to the review and editing of the manuscript and approved the submitted version. N.M. and M.A.M. equal contribution.

Notes

The authors declare no competing financial interest.

Institutional Review Board Statement: The animal study was conducted according to the approved protocol no. DBCOP/IEAC/052020-09 by the Institutional Animal Ethics Committee, Dadasaheb Balpande College of Pharmacy, Besa, Nagpur 440037, Maharashtra, India. The study was conducted according to the guidelines of the Declaration of Helsinki.

■ ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through Large Groups. Project under grant number (RGP.2/207/44).

■ REFERENCES

(1) Montaner, J. S.; Lima, V. D.; Barrios, R.; Yip, B.; Wood, E.; Kerr, T.; Shannon, K.; Harrigan, P. R.; Hogg, R. S.; Daly, P.; Kendall, P. Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study. *Lancet* **2010**, *376* (9740), 532−539.

(2) Günthard, H. F.; Saag, M. S.; Benson, C. A.; del Rio, C.; Eron, J. J.; Gallant, J. E.; Hoy, J. F.; Mugavero, M. J.; Sax, P. E.; Thompson, M. A.; Gandhi, R. T.; Landovitz, R. J.; Smith, D. M.; Jacobsen, D. M.; Volberding, P. A. Antiretroviral Drugs for Treatment and Prevention of HIV Infection in Adults: 2016 Recommendations of the International Antiviral Society-USA Panel. *JAMA* **2016**, *316* (2), 191−210.

(3) Wassner, C.; Bradley, N.; Lee, Y. A Review and Clinical Understanding of Tenofovir: Tenofovir Disoproxil Fumarate versus Tenofovir Alafenamide. *JIAPAC* **2020**, *19*, 232595822091923.

(4) Kearney, B. P.; Flaherty, J. F.; Shah, J. Tenofovir disoproxil fumarate: clinical pharmacology and pharmacokinetics. *Clin. Pharmacokinet.* **2004**, *43* (9), 595−612.

(5) Salawi, A. Self-emulsifying drug delivery systems: a novel approach to deliver drugs. *Drug Delivery* **2022**, *29* (1), 1811−1823.

(6) Neslihan Gursoy, R.; Benita, S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomed. Pharmacother.* **2004**, *58* (3), 173−182.

(7) Rahman, M. A.; Hussain, A.; Hussain, M. S.; Mirza, M. A.; Iqbal, Z. Role of excipients in successful development of self-emulsifying/microemulsifying drug delivery system (SEDDS/SMEDDS). *Drug Dev. Ind. Pharm.* **2013**, *39*, 1−19.

(8) Gao, P.; Jiang, Z.; Luo, Q.; Mu, C.; Cui, M.; Yang, X. Preparation and evaluation of self-emulsifying drug delivery system (SEDDS) of cepharanthine. *AAPS PharmSciTech* **2021**, *22* (7), 245.

(9) Ashour, H. K.; Belal, T. S. New simple spectrophotometric method for determination of the antiviral mixture of emtricitabine and tenofovir disoproxil fumarate. *Arabian J. Chem.* **2017**, *10* (2), S1741−S1747.

(10) Alam, M. S.; Ali, M. D.; Ahmad, S.; Banu, N.; Ali, M. S.; Alam, N.; Ali, M.; Ansari, M. S.; Shamim, M.; Mujtaba, M. A. Evaluation of wound healing potential and biochemical estimation of sage oil nanoemulsion on animal model. *Pak. J. Pharm. Sci.* **2021**, *34* (4), 1385−1392.

(11) Sirvi, A.; Kuche, K.; Chaudhari, D.; Ghadi, R.; Date, T.; Katiyar, S. S.; Jain, S. Supersaturable self-emulsifying drug delivery system: a strategy for improving the loading and oral bioavailability of quercetin. *J. Drug Delivery Sci. Technol.* **2022**, *71*, 103289.

(12) Patel, M. J.; Patel, N. M.; Patel, R. B.; Patel, R. P. Formulation and evaluation of self-microemulsifying drug delivery system of lovastatin. *Asian J. Pharm. Sci.* **2010**, *5*, 266−275.

(13) Maji, I.; Mahajan, S.; Sriram, A.; Medtiya, P.; Vasave, R.; Khatri, D. K.; Kumar, R.; Singh, S. B.; Madan, J.; Singh, P. K. Solid self emulsifying drug delivery system: Superior mode for oral delivery of hydrophobic cargos. *J. Controlled Release* **2021**, *337*, 646−660.

(14) Nazir, I.; Fürst, A.; Lupo, N.; Hupfauf, A.; Gust, R.; Bernkop-Schnürch, A. Zeta potential changing self-emulsifying drug delivery systems: A promising strategy to sequentially overcome mucus and epithelial barrier. *Eur. J. Pharm. Biopharm.* **2019**, *144*, 40−49.

(15) Fule, R.; Paithankar, V.; Amin, P. Hot melt extrusion based solid solution approach: Exploring polymer comparison, physicochemical characterization and in-vivo evaluation. *Int. J. Pharm.* **2016**, *499* (1−2), 280−294.

(16) Hossain, A.; Nandi, U.; Fule, R.; Nokhodchi, A.; Maniruzzaman, M. Advanced surface chemical analysis of continuously manufactured drug loaded composite pellets. *J. Colloid Interface Sci.* **2017**, *492*, 157−166.

(17) Fule, R.; Dhamecha, D.; Maniruzzaman, M.; Khale, A.; Amin, P. Development of hot melt co-formulated antimalarial solid dispersion system in fixed dose form (ARLUMELT): Evaluating amorphous state and in vivo performance. *Int. J. Pharm.* **2015**, *496* (1), 137−156.

(18) Kohli, K.; Mujtaba, A.; Malik, R.; Amin, S.; Alam, M. S.; Ali, A.; Barkat, M. A.; Ansari, M. J. Development of natural polysaccharide-based nanoparticles of berberine to enhance oral bioavailability: formulation, optimization, ex vivo, and in vivo assessment. *Polymers* **2021**, *13* (21), 3833.

(19) Sentenac, S.; Fernandez, C.; Thuillier, A.; Lechat, P.; Aymard, G. Sensitive determination of tenofovir in human plasma samples using reversed-phase liquid chromatography. *J. Chromatogr. B* **2003**, *793* (2), 317−324.

(20) Ahmad, J.; Kohli, K.; Mir, S. R.; Amin, S. Formulation of self-nanoemulsifying drug delivery system for telmisartan with improved dissolution and oral bioavailability. *J. Dispersion Sci. Technol.* **2011**, *32* (7), 958−968.

(21) Zaichik, S.; Steinbring, C.; Caliskan, C.; Bernkop-Schnürch, A. Development and in vitro evaluation of a self-emulsifying drug delivery system (SEDDS) for oral vancomycin administration. *Int. J. Pharm.* **2019**, *554*, 125−133.

(22) Jadhav, C.; Kate, V.; Payghan, S. A. Investigation of effect of non-ionic surfactant on preparation of griseofulvin non-aqueous nanoemulsion. *J. Nanostruct. Chem.* **2015**, *5*, 107−113.

(23) Sarfaraz Alam, M.; Ali, M. S.; Zakir, F.; Alam, N.; Intakhab Alam, M.; Ahmad, F.; Siddiqui, M. R.; Ali, M. D.; Ansari, M. S.; Ahmad, S.; Ali, M. Enhancement of anti-dermatitis potential of clobetasol propionate by DHA [docosahexaenoic acid] rich algal oil nanoemulsion gel. *Iran. J. Pharm. Res.* **2016**, *15* (1), 35−52.

(24) Azeem, A.; Rizwan, M.; Ahmad, F. J.; Iqbal, Z.; Khar, R. K.; Aqil, M.; Talegaonkar, S. Nanoemulsion components screening and selection: a technical note. *AAPS PharmSciTechnol* **2009**, *10* (1), 69−76.

(25) Suchaoin, W.; Pereira de Sousa, I.; Netsomboon, K.; Lam, H. T.; Laffleur, F.; Bernkop-Schnürch, A. Development and in vitro evaluation of zeta potential changing self-emulsifying drug delivery systems for enhanced mucus permeation. *Int. J. Pharm.* **2016**, *510* (1), 255−262.

(26) Sharifi, F.; Nazir, I.; Asim, M. H.; Jahangiri, M.; Ebrahimnejad, P.; Matuszczak, B.; Bernkop-Schnürch, A. Zeta potential changing self-emulsifying drug delivery systems utilizing a novel Janus-headed surfactant: A promising strategy for enhanced mucus permeation. *J. Mol. Liq.* **2019**, *291*, 111285.

(27) Abou Assi, R.; M Abdulbaqi, I.; Seok Ming, T.; Siok Yee, C.; A Wahab, H.; Asif, S. M.; Darwis, Y. Liquid and Solid Self-Emulsifying Drug Delivery Systems (SEDDs) as Carriers for the Oral Delivery of Azithromycin: Optimization, In Vitro Characterization and Stability Assessment. *Pharmaceutics* **2020**, *12* (11), 1052.

(28) Zaichik, S.; Steinbring, C.; Menzel, C.; Knabl, L.; Orth-Höller, D.; Ellemunter, H.; Niedermayr, K.; Bernkop-Schnürch, A. Development of self-emulsifying drug delivery systems (SEDDS) for ciprofloxacin with improved mucus permeating properties. *Int. J. Pharm.* **2018**, *547*, 282−290.

https://doi.org/10.1021/acsomega.3c08565
ACS Omega 2024, 9, 8139−8150

(29) Mahmood, A.; Khan, L.; Ijaz, M.; Nazir, I.; Naseem, M.; Tahir, M. A.; Aamir, M. N.; Rehman, M. U.; Asim, M. H. Enhanced intestinal permeability of cefixime by self-emulsifying drug delivery system: in-vitro and ex-vivo characterization. *Molecules* **2023**, *28* (6), 2827.
(30) Cardona, M. I.; Dominguez, G. P.; Echeverry, S. M.; Valderrama, I. H.; Bernkop-Schnürch, A.; Aragón, M. Enhanced oral bioavailability of rutin by a self-emulsifying drug delivery system of an extract of calyces from Physalis peruviana. *J. Drug Delivery Sci. Technol.* **2021**, *66*, No. e102797.
(31) Balakrishnan, P.; Lee, B.-J.; Oh, D. H.; Kim, J. O.; Lee, Y.-I.; Kim, D.-D.; Jee, J.-P.; Lee, Y.-B.; Woo, J. S.; Yong, C. S.; Choi, H.-G. Enhanced oral bioavailability of Coenzyme Q10 by self-emulsifying drug delivery systems. *Int. J. Pharm.* **2009**, *374* (1−2), 66−72.

https://doi.org/10.1021/acsomega.3c08565
ACS Omega 2024, 9, 8139−8150