RESEARCH PAPER

Formulation and Optimization of Tadalafil Nanosuspension Using High Shear Homogenization and Design of Experiments Approach for Solubility and Bioavailability Enhancement

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ABSTRACT

Tadalafil (TDF), acting as a phosphodiesterase-5 inhibitor and clinically used for benign prostatic hyperplasia, pulmonary arterial hypertension, and erectile dysfunction suffers from poor aqueous solubility and low oral bioavailability. In this research investigation, a stable nanosuspension (NS) of Tadalafil (TDF) was developed and optimized using high-shear homogenization (HSH) within a Quality by Design (QbD) framework. Box–Behnken design assessed the effects of drug-to-stabilizer ratio, homogenization speed, and time on particle size, PDI, and drug loading. The optimized NS showed a particle size of 515.9 ± 42 nm, PDI 0.539 ± 0.034, and drug loading around 70%.Differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD) confirmed partial amorphization of TDF. FE-SEM analysis revealed spherical nanosized particles. Dissolution studies demonstrated >90% release within 60 min compared with 20% for pure TDF suspension. Stability testing under accelerated conditions confirmed physical stability for 3 months. Pharmacokinetic evaluation in Wistar rats revealed significantly higher Cmax and AUC for NS, corresponding to ~2.5-fold improvement in relative oral bioavailability versus drug suspension. This work demonstrates that HSH is a robust, scalable approach for improving solubility, dissolution, and bioavailability of tadalafil.

Keywords: Tadalafil; nanosuspension; high shear homogenization; solubility enhancement; Box–Behnken design; pharmacokinetics

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1. INTRODUCTION

Oral drug absorption is often hindered by poor aqueous solubility—a challenge particularly acute for Biopharmaceutics Classification System (BCS) Class II compounds, which exhibit low solubility but high permeability [1] . This dissolution-limited absorption often culminates in suboptimal bioavailability, impeding therapeutic efficacy. Moreover, a significant portion of new chemical entities are hindered by this issue; estimates suggest that between 40% and 70% of novel drugs suffer from low solubility, with only around 8% displaying both high solubility and permeability. To surmount solubility and dissolution barriers, nanosuspensions have emerged as a powerful formulation strategy. These dispersions consist of drug particles suspended in an aqueous medium, stabilized by suitable surfactants or polymers, without requiring matrix materials [2] . By reducing particle size to submicron range, nanosuspensions dramatically increase surface-area, thereby enhancing dissolution rates as per the Noyes-Whitney equation. Additionally, saturation solubility itself can rise due to elevated surface energy and altered crystalline form, as described by the Ostwald–Freundlich relationship [3] .TDF useful clinically in treating erectile dysfunction, pulmonary arterial hypertension and benign prostatic hyperplasia [4] . Despite its favourable pharmacological profile its therapeutic performance is compromised by poor water solubility and dissolution rate [5] . These properties classify it as a BCS Class II drug, where dissolution is the rate-limiting step for absorption [6] .

In this study, we aim to develop and optimize a TDF NS using HSH as a scalable top-down technique paired with Design of Experiments (DoE) to systematically probe and optimize critical formulation and process parameters. This framework aligns with QbD principles, fostering robust, reproducible, and high-performance formulations.

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Although TDF has been investigated in various contexts, there remains a paucity of systematic research integrating QbD-oriented formulation design, DoE-based optimization, and in vivo validation of TDF nanosuspensions. This work fills that gap by combining methodical optimization with pharmacokinetic evaluation, thereby advancing toward translational viability.

2. MATERIALS AND METHODS

2.1 Materials

Tadalafil (TDF), hydroxypropyl methylcellulose (HPMC E15), polyvinylpyrrolidone K30 (PVP-K30), and polyvinyl alcohol (PVA) were kindly provided by Zim Laboratories Ltd., Kalmeshwar, Nagpur, India. D-α-Tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS) was received from Matrix Fine Sciences Pvt. Ltd., Aurangabad, India. Poloxamer 188, poloxamer 407, sodium lauryl sulfate (SLS), and polyethylene glycol 400 (PEG 400) were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Tween 80 was purchased from Merck India Ltd. All other reagents and chemicals were of analytical grade.

2.2 Preliminary Screening of Parameters for NS Preparation

Initial screening of formulation and process parameters included type and concentration of stabilizer, homogenization speed, and homogenization time using a one-variable-at-a-time (OVAT) approach. Stabilizers from various classes were evaluated: non-ionic surfactants (poloxamer 188, poloxamer 407, Tween 80), polymeric stabilizers (PVP-K30, HPMC E15, PVA), anionic surfactant (SLS), and amphiphilic stabilizer (Vitamin E TPGS). Stabilizer selection was based on CUR solubility in the stabilizer solution (shake-flask method) and wettability improvement (contact angle measurement). Different drug: stabilizer ratios 1:1, 1:2.5, 1:5 and 1:10 were screened. NS were formulated with varying homogenization speeds from 5000 to 15000 rotation per minute (rpm) and homogenization time from 120 to 240 mins.

2.3 Preparation of TDF NS

A High shear homogenizer (Polytron PT 3100 D, Kinematica) was used to carry out the process of size reduction. An accurately weighed amount of TDF was added to the stabilizer solution containing Poloxamer 188 . The resultant liquid mixture of drug and stabilizer was subjected to HSH to yield NS. [7,8]

2.4 DoE based optimization of TDF NS

A Box-Behnken (BB) statistical design [9, 10, 11, 12] was used to study the influence of selected independent variables on measured responses (dependent variables). Drug: stabilizer ratio (A), homogenization speed (B) and homogenization time (C) were identified as independent variables whereas average-particle size (PS), Poly Dispersity Index (PDI) and drug loading were selected as dependent variables (responses). A three-factor, three-level design was employed. The list of independent parameters and their selected levels is given in Table 1. Other factors such as type of stabilizer (Poloxamer 188), amount of drug, solvent (distilled water) and volume (100 ml) were kept constant for all the experiments. Fifteen formulation batches were designed by taking three centre points per block. The design matrix and corresponding formulation code are shown in Table 2.

Table 1: The level of variables in the BB design

Coded Factors	Low (-1)	Medium (0)	High (+1)
Amount of surfactant (Drug:Stabilizer ratio) A	1:2.5	1:5.0	1:7.5
Homogenization (H.) Speed (rpm) B	10000	12500	15000
Homogenization (H.) Time (min) C	60	120	180

Table 2: Design matrix for preparation of TDF NS

Run no.	Formulation code	Independent variable		
		Amount of surfactant (A) [Drug: Stabilizer ratio]	H. Speed (RPM) (B)	H. Time (min) (C)
1.	T1	1:5	15000	180
2.	T2	1:7.5	10000	120
3.	T3	1:7.5	12500	180
4.	T4	1:2.5	15000	120
5.	T5	1:7.5	12500	60
6.	T6	1:2.5	12500	60
7.	T7	1:2.5	10000	120
8.	T8	1:5	10000	60
9.	T9	1:5	15000	60
10.	T10	1:2.5	12500	180
11.	T11	1:5	10000	180
12.	T12	1:5	12500	120
13.	T13	1:5	12500	120
14.	T14	1:7.5	15000	120
15.	T15	1:5	12500	120

2.5 Data analysis, statistical optimization and model validation

The experimental data were analyzed using multiple linear regression to fit them into an appropriate polynomial model, incorporating interaction terms to establish correlations between the investigated variables and responses with the aid of Design Expert® software (Version 7, Stat-Ease Inc., Minneapolis). The statistical significance of the developed polynomial models was confirmed through analysis of variance (ANOVA). Response surface methodology was applied using two-dimensional contour plots and three-dimensional response surface plots to visualize the effects. Numerical optimization was employed to identify the most suitable formulation, with the desirability function targeted to approach 1. To verify the reliability of the DoE approach, three checkpoint formulations were prepared and assessed. [11,10,13]

2.6 Characterization of NS

2.6.1 Particle size analysis

It was carried out using photon correlation spectroscopy using Zetasizer (Nano ZS 90, Malvern Instruments Ltd., Malvern, UK) for determining average particle size and PDI. All the samples were diluted with filtered bidistilled water (1:10) to get optimum kilo-counts/s (kcps) for measurement. All the measurements were performed at 25ºC and scattering angle of 90º. [14, 15] The determinations were carried out in triplicate and results were expressed as mean±SD.

2.6.2 Drug loading

An aliquot (1ml) of NS was diluted up to 10 ml with methanol and filtered. The sample was analyzed spectrophotometrically at λmax 284 nm using methanol as a blank. All the determinations were carried out in triplicate and results expressed as mean±SD. [16, 17]

2.6.3 Zeta potential

Zeta potential (ζ) was also determined by photon correlation spectroscopy using Zetasizer (Nano ZS 90, Malvern Instruments Ltd., Malvern, UK). The measurements were run at an electric field strength of 25 V/m. The studies were done on optimized NS formulation. The determinations were carried out in triplicate and results were expressed as mean±SD. [15]

2.6.4 Saturation solubility studies

It was carried out for both the pure drug and optimized NS. An accurately weighed amount of pure drug (10 mg) and NS equivalent to 10 mg of drug were separately introduced into a stoppered conical flask (25 ml) containing 10 ml of distilled water. The flasks were sealed and placed in a rotary shaker at 37 °C for 48 hours. The contents were then filtered, and the suitably diluted samples were analyzed using a UV-Visible spectrophotometer (Shimadzu-1800, Japan) at 284 nm, against distilled water as a blank. The determinations were carried out in triplicate and results were expressed as mean±SD. [8,18]

2.6.5 Fourier Transform Infrared Spectroscopy (FTIR):

Infrared spectrums of pure drug, excipients and drug-excipient physical mixture were recorded using FTIR (Shimadzu FTIR-8101) according to the KBr disk method.

2.6.6 Differential scanning calorimetry (DSC)

DSC analysis was performed on the pure drug, physical mixture of drug and excipient and optimized NS formulation. Accurately weighed samples (2-4 mg) were placed in hermetically sealed aluminium pans. An empty pan was used as a reference in an inert atmosphere under constant nitrogen purge. Thermograms were obtained by Differential Scanning Calorimeter (DSC 1, Mettler-Toledo, Zurich, Switzerland) at a heating rate of 10^0C/min from 30^0C to 300^0C under a nitrogen purge of 40 ml/min. [15,19]

2.6.7 X-Ray Powder Diffraction (XRPD)

The XRPD analysis of TDF API and TDF NS was recorded using an X-ray diffractometer (Rigaku Smartlab, Japan). The pure drug powder and a dried sample of optimized NS were irradiated with monochromatized Cu Kα radiation (1.542 A) and analyzed between 5 and 50° 2θ angle. [20,21]

2.6.8 Particle morphology

The morphology of TDF and its nanocrystals was evaluated through a field emission scanning electron microscope (Supra 55, Carl Zeiss, Germany). Before analysis, the prepared TDF NS was dropped on a glass slide, and dried with an infrared lamp. The dried sample was fixed on an SEM stub using double-sided adhesive tape and then coated with a thin layer of gold under a vacuum. The structures of the examined samples were observed and compared. [21]

2.6.9 In vitro dissolution study

In vitro dissolution studies were performed for plain TDF pure powder (10 mg), and an equivalent amount of optimized TDF NS. The samples were dispersed in a 900 ml aqueous solution containing 0.5% (w/v) Sodium Lauryl Sulphate using USP dissolution apparatus II at 50 rpm and 37±0.5°C. Aliquots were periodically withdrawn (5 ml), filtered and analysed spectrophotometrically at 284 nm and were replaced with an equal volume of dissolution medium. The determinations were carried out in triplicate and results were expressed as mean±SD. [16]

2.6.10 Stability studies

The final optimized NS formulation was kept at an accelerated temperature and relative humidity conditions (40±2 ºC and 75±5% RH) for 3 months. Periodically, samples were withdrawn at an interval of 1, 2 and 3 months and subjected to particle size analysis and per cent drug loading of the same. The determinations were carried out in triplicate and results were expressed as mean±SD. [20,19,22]

2.6.11 In vivo pharmacokinetic (PK) study

The protocol of the animal study was approved by the institutional animal ethical committee

(Letter no.: PH/IAEC/VNS/2K21/09). All the in vivo experiments were carried out following the ethical guidelines for the use and care of animals.

Healthy Albino Wistar rats, with body weights ranging between 150 and 200 g were used and fasted overnight

before the study. A single-dose PK study was designed involving two groups (Group I and II) (n=6) Samples were administered using an oral feeding cannula. Blood samples (0.5 ml) were collected from the retroorbital plexus of rats at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24 and 48 hours post-dosing and placed in heparinized Eppendorf tubes. Samples were centrifuged at 10000 rpm for 10 min at 2-4ºC and plasma was separated and collected. To 200 µl of rat plasma collected at each study point, 300 µl of acetonitrile was added and centrifuged at 10000 rpm for 10 min. The supernatant was collected and analyzed under the optimized chromatographic conditions. The Peak area of the drug was recorded and the concentrations of the drug were calculated using the regression equation (21)(9). PK analysis of plasma drug concentration data was conducted employing excel add-in PK solver 2.0. PK parameters like elimination half-life (t½), maximum observed plasma concentration during the study period (C_{max}) and the corresponding time (T_{max}), and area under the curve (AUC_{0-t} and $AUC_{0-\infty}$) were computed. The relative bioavailability (F_{rel}) of formulations was measured by taking the ratio of AUC of NS and AUC of pure drug suspension. [19,20]

3. RESULT & DISCUSSION

3.1 Preliminary screening of parameters

Considering, the main desired quality attributes of NS i.e. particle size distribution and drug loading; significant factors namely type and amount of surfactant, homogenization speed and time were selected for further studies. Saturation solubility and contact angle measurements are the most reliable tools for selecting appropriate stabilizer for preparing NS. [23] Therefore, the initial screening of the stabilizers was carried out based on these two methods. Poloxamer 188 was found to be ideal on the basis of saturation solubility data and contact angle measurements, and hence shortlisted for further studies. Poloxamers are amphiphilic block polymers, containing both hydrophobic and hydrophilic chains providing both steric hindrance for preventing particle aggregation and adsorption on the crystal surface. [24]

3.2 DoE based optimization of TDF NS

In this study, DoE was applied to evaluate the influence of Critical Material Attributes (CMAs), such as stabilizer concentration, along with Critical Process Parameters (CPPs) including homogenization speed and duration, as well as their interactions, on the Critical Quality Attributes (CQAs) of the nanosuspension. Particle size, polydispersity index (PDI), and drug loading were selected as the key CQAs representing the overall formulation quality. To predict the optimal formulation, a BBD was employed. The impact of stabilizer level, homogenization rate, and processing time on the CQAs of tadalafil nanosuspension was systematically examined. The BBD, structured as a three-factor, three-level (3^3) design incorporating replicated central points and edge midpoints of the experimental cube, efficiently defines the study domain. Compared to central composite and full factorial designs, BBD is advantageous as it reduces the number of experimental trials required for process optimization. [25]

3.3 Data analysis, statistical optimization and model validation

The observed responses for the designed experimental runs are presented in Table 3. A quadratic model was selected for analyzing all the responses. The responses Y_1 (Particle size), Y_2 (PDI) and Y_3 (Drug loading) ranged from 547.2 nm to 1544.2 nm, 0.08 to 0.91 and 24 to 73 respectively. The design summary is given in Table 4. Analysis of variance (ANOVA) was applied to study the importance of variables on the responses

.

Table 3: BBD: Composition of variables and recorded responses

Run no.	Formulation code	Independent variables			Responses		
		Amount of surfactant (Drug: Stabilizer ratio) A	Homo. Speed (RPM) B	Homo. Time (min) C	Particle size (nm) Y_1	PDI Y_2	% Drug loading Y_3
1.	T1	1:5	15000	180	617	0.08	60
2.	T2	1:7.5	10000	120	1480	0.91	28.3
3.	T3	1:7.5	12500	180	1226.4	0.618	32.6
4.	T4	1:2.5	15000	120	669.2	0.385	69
5.	T5	1:7.5	12500	60	1544.2	0.817	24
6.	T6	1:2.5	12500	60	990.7	0.446	51
7.	T7	1:2.5	10000	120	995.1	0.364	53
8.	T8	1:5	10000	60	776.2	0.497	54
9.	T9	1:5	15000	60	659.4	0.216	56
10.	T10	1:2.5	12500	180	547.2	0.332	73
11.	T11	1:5	10000	180	756.8	0.349	55
12.	T12	1:5	12500	120	650	0.414	58.1
13.	T13	1:5	12500	120	667.6	0.397	57.5
14.	T14	1:7.5	15000	120	1377	0.467	31
15.	T15	1:5	12500	120	638.3	0.516	58.4

Table 4: Design summary of responses

Response	Name	Units	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
Y_1	Particle size	nm	Polynomial	547.2	1544.2	906.3	330.5	2.822	None	Quadratic
Y_2	PDI	……	Polynomial	0.08	0.91	0.453	0.202	11.37	None	Quadratic
Y_3	Drug loading	%	Polynomial	24	73	50.72	14.31	3.041	None	Quadratic

Particle Size:

Reduction in particle size enhances surface area, leading to improved saturation solubility of poorly soluble drugs. [26] Accordingly, particle size was identified as a key CQA for TDF NS. Across 15 designed experiments, particle sizes varied between 547.2 and 1544.2 nm. The selected 'quadratic' model was significant with a significant lack of fit. The model F value obtained was 13.34 which implies that there is a 0.54% chance that it may be due to noise. The p values of the model term A and A^2 were found to be < 0.05 indicating their significant role on the response. Model term B was found to be insignificant with a p-value > 0.05 however model term C was a little close to significance with a p-value of 0.0517. The regression analysis in coded terms generated the following equation for particle size:

Particle size= +652+303*A-85.6 *B-102* C+55.7 *AB+31.4 *AC-5.75 *BC+426 *A^2+51.7 *B^2-1.40 *C^2

Where A is the amount of surfactant, B is Homogenization speed and C is homogenization time. A, B, and C are linear terms. AB, BC, and AC are interaction terms and A^2, B^2 and C^2 represents quadratic terms. A positive sign indicates synergistic effect, while a negative sign denotes antagonistic effect. [27] The amount of surfactant played major role on the particle size of NS. The different drug: stabilizer ratios studied were 1:2.5, 1:5 and 1:7.5. At low and medium levels of surfactant amount, the particle size was below 1000 nm, whereas, an increase in the concentration of surfactant led to increased particle size. An insufficient quantity of stabilizer may lead to accelerated crystal growth due to inadequate surface coverage, whereas an excessive amount can enlarge particle size by forming a thicker protective layer. [12] Also, increased viscosity of the aqueous medium at a higher amount of surfactant might have led to a reduction in the net shear stress at constant energy input for particle breakdown. Hence, the particle size increased with an increase in surfactant amount. Both the process parameters viz homogenization speed and time affected the particle size of NS. An increase in both homogenization speed and time has led to a reduction in particle size. This may be due to increased and continuous shear produced with incremental speed and time of homogenization leading to enhanced breakage kinetics. [28]

PDI: It is the measure of distribution of particles in the dispersion (broadness of size). It typically ranges from 0 to 1; however, if the obtained value is > 0.5, the sample is considered to have a broader distribution of particles (hetero-disperse) whereas <0.5 indicates a monodisperse system. [26] Broader distribution of particles accounts for Ostwald ripening; responsible for poor long term stability. The PDI of the developed formulations was in the range of 0.08 to 0.91. The selected 'quadratic' model was significant with an insignificant lack of fit. The model F value obtained was 31.73 which implies that the model is significant and there is only a 0.07% chance that it may be due to noise. The predicted p values of the model term A, B, C, AB, A^2, B^2 and C^2 were found to be < 0.050 indicating their significant role on the response. The following equation was generated for PDI :

PDI=0.442+0.160*A-0.121*B-0.074*C-0.116*AB-0.021*AC+0.003*BC+0.178*A^2-0.089*B^2-0.067*C^2

Which shows the synergistic effect of the amount of surfactant on PDI i.e., an increase in surfactant amount also increased the value of PDI. While an antagonistic effect is seen with both homogenization time and speed. As both of these factors increased, the PDI decreased indicating a shift towards a more monodisperse system. As evident from the effect of surfactant amount on particle size, excess or higher amount of surfactant is also not suitable for attaining lower values of PDI. Homogenization speed and homogenization time are one of the important strategies for applying kinetic energy to achieve lower particle size and PDI. An increase in both homogenization speed and time has resulted in increased shearing effects causing a decrease in the dispersity index and thereby creating a more homogenous system. [29]

Drug loading: Drug loading refers to the quantity of drug that has been successfully reduced in size and remains in the supernatant after homogenization. In many cases, however, the efficiency of dispersion achieved by HSH can be adversely affected by the presence of microparticles. [29] Hence, this study was also focused to overcome this disadvantage and maximize the amount of drug present as nanocrystals. The per cent drug loading of the developed formulations was in the range of 24 % to 73 %. The model F value obtained was 16.14 which implies that the model is significant and there is only a 0.35% chance that it may be due to noise. The p values of the model term A, C, and A^2 were found to be < 0.05 indicating their significant role on the response. Following equation was generated for drug loading:

Drug loading = 58-16.2*A+3.21*B+4.45*C-3.32*AB-3.35*AC+0.75*BC-11.8*A^2-0.787*B^2-0.962*C^2

The amount of surfactant has an antagonistic effect on the drug loading whereas both homogenization speed and time have synergistic effects. At a higher concentration of surfactant, the drug loading was minimum because a high amount of surfactant could not effectively reduce the particle size and also exhibited a high degree of polydispersity. On the contrary, high drug loading was observed at a lower concentration of surfactant where the average particle size was in the range of 500 nm with a PDI of <0.5. As the homogenization speed and time increased, the drug loading also increased. Homogenization time was found to be a more significant factor that affected drug loading. Longer processing times (homogenization time) increased the drug loading of NS.

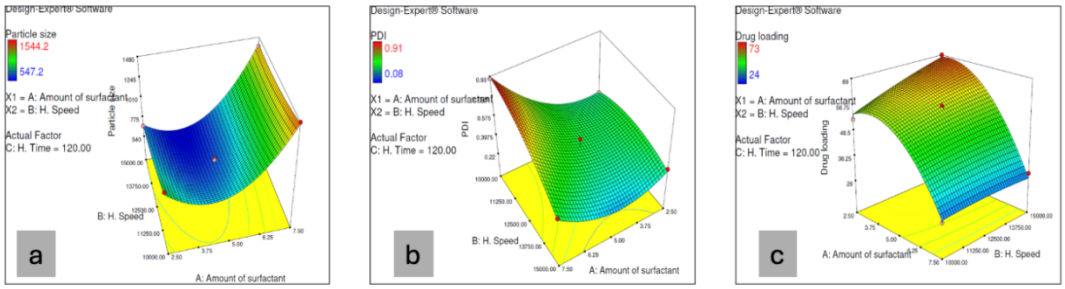

Figure 1: Response surface plots illustrating the effect of amount of surfactant and homogenization speed on a) Particle size b) PDI c) Drug Loading

Numerical optimization using the desirability function highlighted a formulation with the highest score of 0.945 as the most favourable (Figure 2). This optimized NS was achieved with a drug-to-stabilizer ratio of 2.51 (1.25 g Poloxamer 188 per 500 mg drug), homogenized at 15,000 rpm for 180 minutes. The selection was based on achieving a balance of minimal particle size and PDI, high drug loading, and the lowest effective stabilizer concentration.

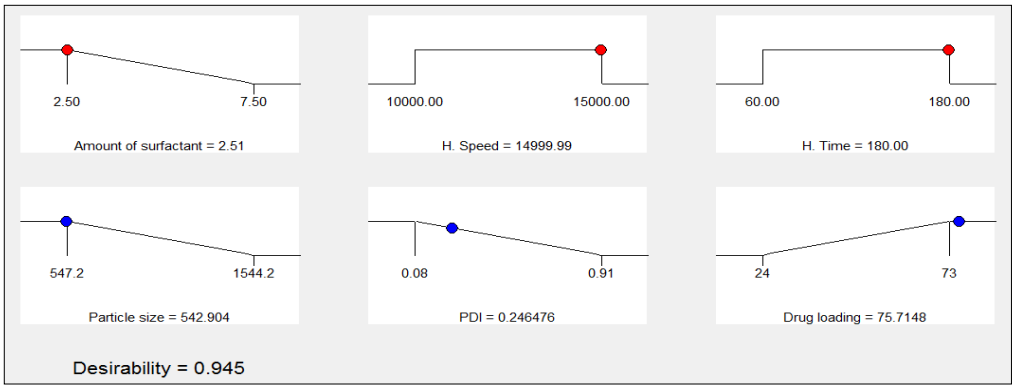

Figure 2: Numerical optimization using desirability function for NS of the model drug TDF

Model validation was performed using three checkpoint formulations. Predicted values for particle size, PDI, and drug loading were 542.9 nm, 0.246, and 75.7%, respectively, while observed values were 515.9 ± 42 nm, 0.539 ± 0.034, and 70 ± 1.2%, demonstrating good agreement and confirming the model's reliability.

3.4 Characterization of NS

3.4.1 Particle size analysis

The average particle size of the optimized NS formulation of model drug TDF is shown in Figure 3. The average particle size was found to be 515.9±42 nm with a PDI 0.539±0.034. Although, HSH could easily reduce the particle size to submicron range, the optimized formulation exhibited a PDI of 0.54, indicating relatively broader particle size distribution. Such a PDI value reflects heterogeneity within the dispersion, with the coexistence of both smaller and larger particles. This broader distribution may influence the physical stability of the NS, as non-uniform systems are more susceptible to particle growth, aggregation, and Ostwald ripening over time. The observed value can be attributed to the current processing conditions. Unlike high-pressure homogenization, where the entire sample passes through the same restrictive path, high shear processing involves different treatment intensities for different parts of the sample which may possibly lead to non-uniform size reduction. Even though the formulation achieved nanoscale particle size with enhanced solubility and dissolution, it is desirable to obtain a monodisperse system for long-term stability. Further optimization of homogenization parameters and, or the use of stabilizer combinations, may help in narrowing the size distribution.

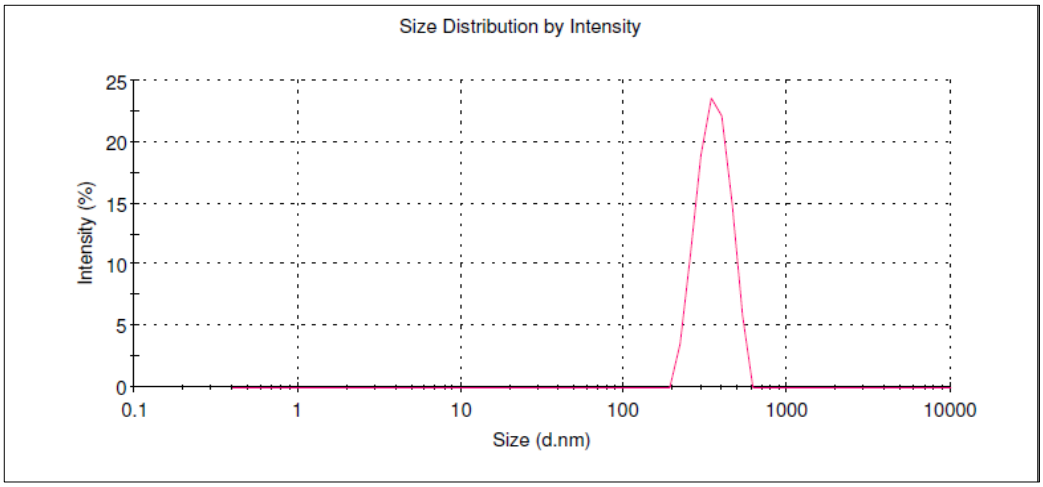

Figure 3: Particle size analysis data of optimized NS of TDF

3.4.2 Drug loading

The optimized NS reported 70±1.2 % drug loading.

3.4.3 Zeta potential

The charge on the surface of particles is characterized by measuring the zeta potential of a suspension. The zeta potential of the sample is most often used as an indicator of dispersion stability. It is essential both for the formation of NS and storage. (30) Optimized NS showed a zeta potential value of -6.76 mV±2.4 (Figure 4). NS formulation was stabilized using a non-ionic stabilizer, Poloxamer 188; which imparts steric stabilization by increasing the diffuse double layer thickness at the surface thereby lowering the zeta potential. [30] Hence, in this case, the stability is mostly steric due to Poloxamer 188. [20]

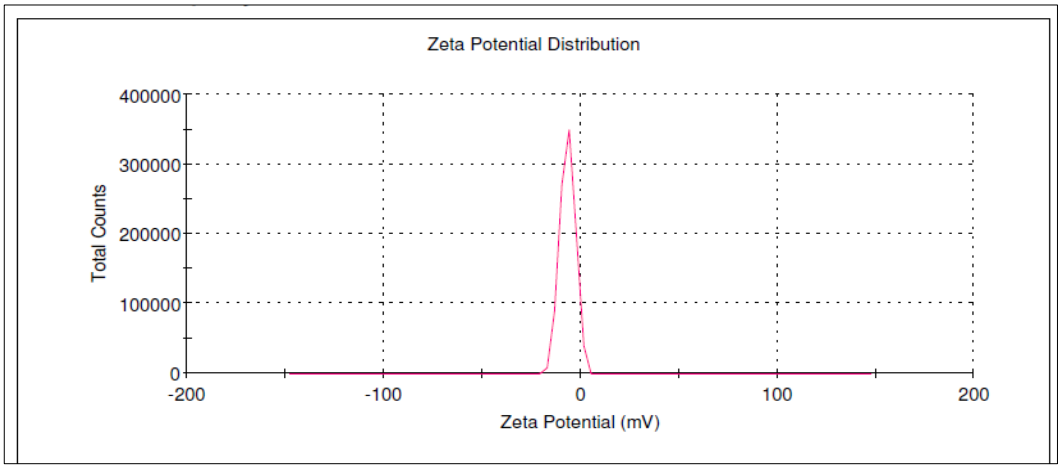

Figure 4 : Zeta potential data of optimized NS of TDF

3.4.4 Saturation solubility studies

The optimized TDF NS exhibited a saturation solubility of 370 µg/mL, compared to 6.12 µg/mL for the pure drug, representing a 60-fold increase. This enhancement is attributed to particle size reduction and the resulting increase in surface area, which, according to the Ostwald–Freundlich equation, improves solubility and can significantly enhance oral bioavailability. [20,26]

3.4.5 FTIR Spectroscopy

The main characteristic peaks of pure TDF were 774 cm^{-1} (benzene), 922 cm^{-1} (O-H bending), 1042 cm^{-1} (C-O-C stretching), 1402 cm^{-1} (C-O stretching), 1653 cm^{-1} (C=C aromatic), 1676 cm^{-1} (C=O), 2904 cm^{-1} (C-H stretching) and 3329 cm^{-1} (Aromatic O-H stretching). [31,17,21,16,9]

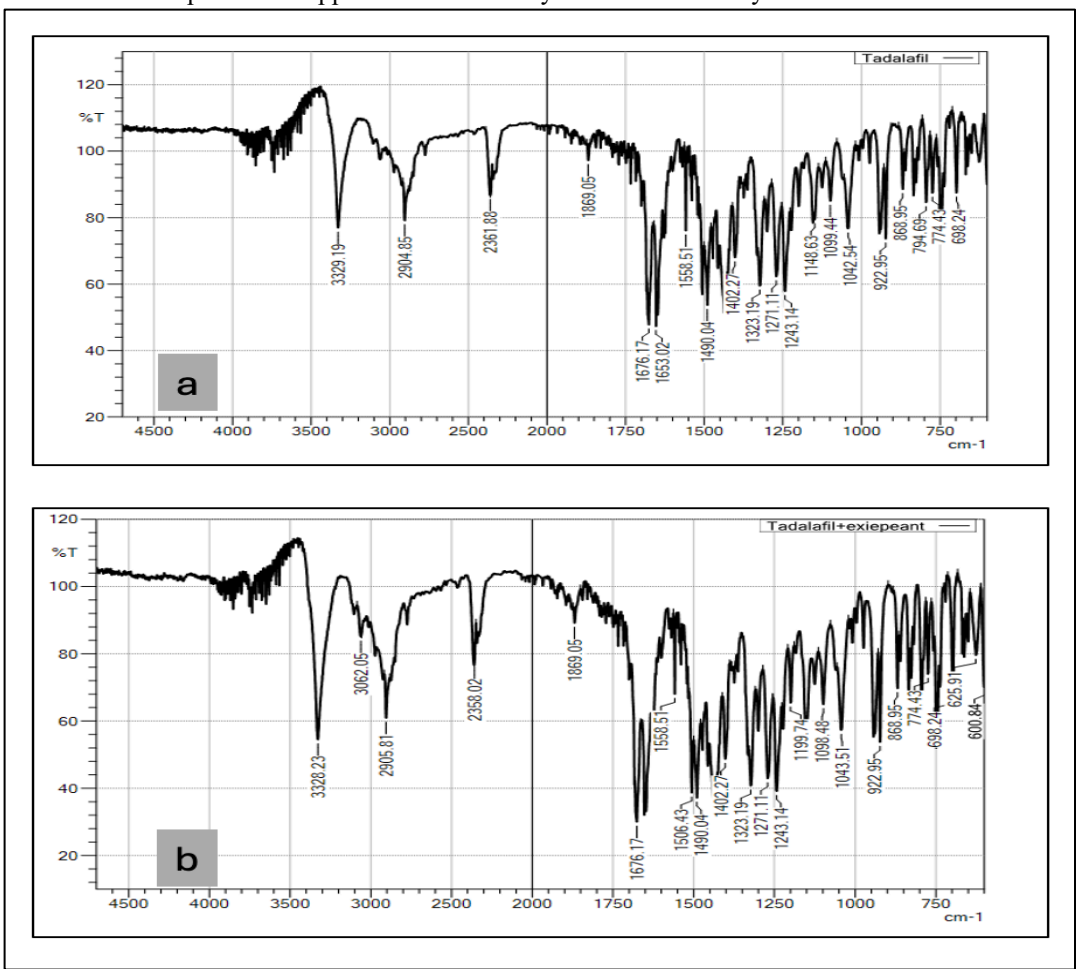

Figure 5 : a) FTIR spectra of TDF b) FTIR spectra of physical mixture of TDF and excipients

FTIR spectra of the pure TDF and the physical mixture (Figure 5a and 5b) were almost similar in peak pattern and frequency indicating absence of any significant interaction between the drug and studied excipients.

3.4.6 DSC

DSC was performed to investigate the changes in crystallinity of TDF before and after the size reduction process and the obtained results were assessed by comparing the DSC thermograms of pure drug, physical mixture of drug with stabilizer and NS. As shown in Figure 6 a , the DSC thermogram of TDF exhibited a single, sharp endothermic peak at 300.57º C, which corresponds to the melting point of TDF and indicates that TDF was present in a crystalline state. The Thermogram of the physical mixture (Figure 6 b) exhibits the presence of individual melting peaks of the drug and stabilizer. However, the thermogram of NS reveals a change in the melting point of TDF as compared to that of pure TDF (Figure 6 c) and is decreased to around 100º C from 300º C. The shifts towards lower values could be attributed to the formation of some drug-stabilizer complexes as a result of intermolecular interactions between the drug and excipients. Thus, the reduced melting point in this context could be possibly due to excipient interference.[31,17,21,16]

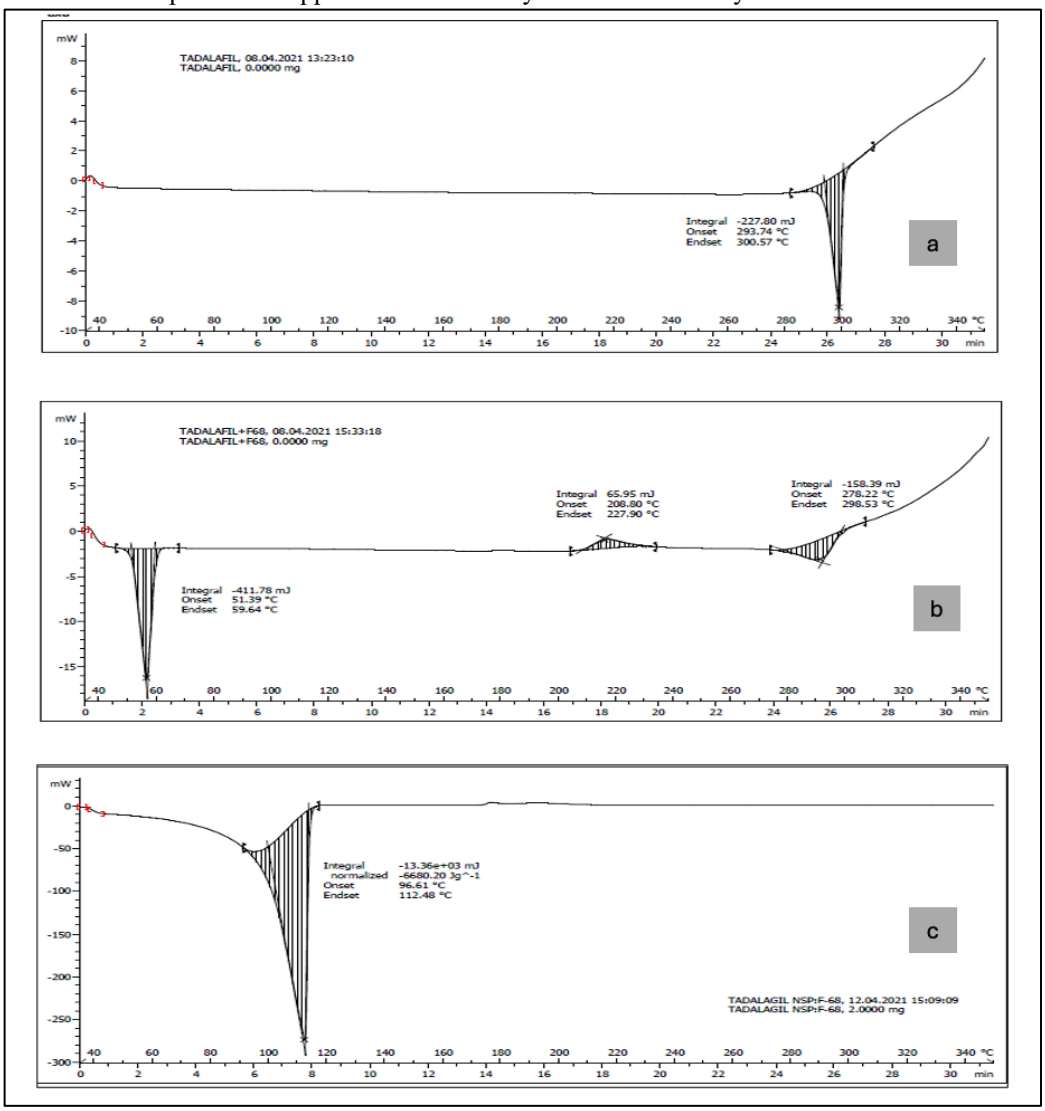

Figure 6 : a) DSC thermogram of pure TDF b) DSC thermogram of Physical mixture of TDF and excipient c) DSC thermogram of TDF NS

3.4.7 XRPD

The X-ray diffraction spectra of pure TDF drug and lyophilized NS are illustrated in Figure 7a and 7b. The XRD spectrum of TDF displayed sharp peaks at 2θ diffraction angles of 7.3^0, 9.7^0, 14.7^0, 18.8^0, 20.5^0, 23.5^0. The diffraction pattern of lyophilized NS manifested several sharp high intensity peaks at similar diffraction angles (2θ values) signifying that the drug existed as a crystalline material. There was no major difference in the relative intensities of peaks indicating minute changes in the original crystalline state of the drug. [31, 17,21, 16,9] Hence, it can be manifested that the formation of NS by HSH does not alter the original form of the drug and retains structural integrity implicating the physical stability of the nano formulation and avoidance of any compromised behavioural characteristics of the drug.

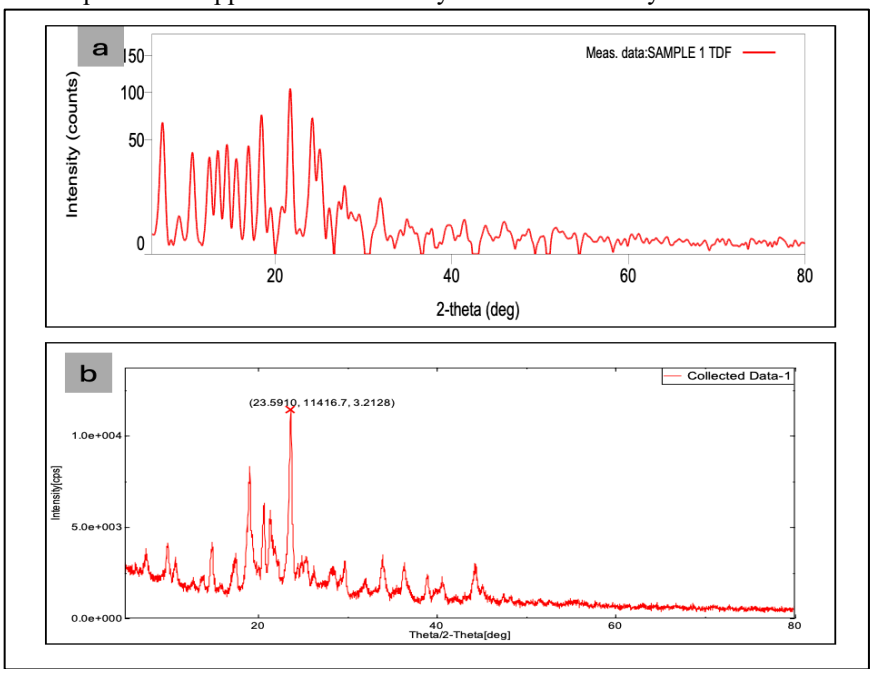

Figure 7: a) Diffractogram of TDF b) Diffractogram of lyophilized NS

3.4.8 Particle morphology

FE-SEM image of the pure TDF and TDF NS is shown in Figure 8 (a) and (b) on a scale of 10 µm. The unprocessed pure TDF exhibited irregularly shaped large acicular crystals with a non-uniform particle size distribution. On the contrary, whereas, TDF in NS form has smaller and more uniform particles. Although, there is no change in the shape of particles post size reduction the size has reduced considerably as visible in the images. The results corroborate the data obtained for particle size distribution for NS.

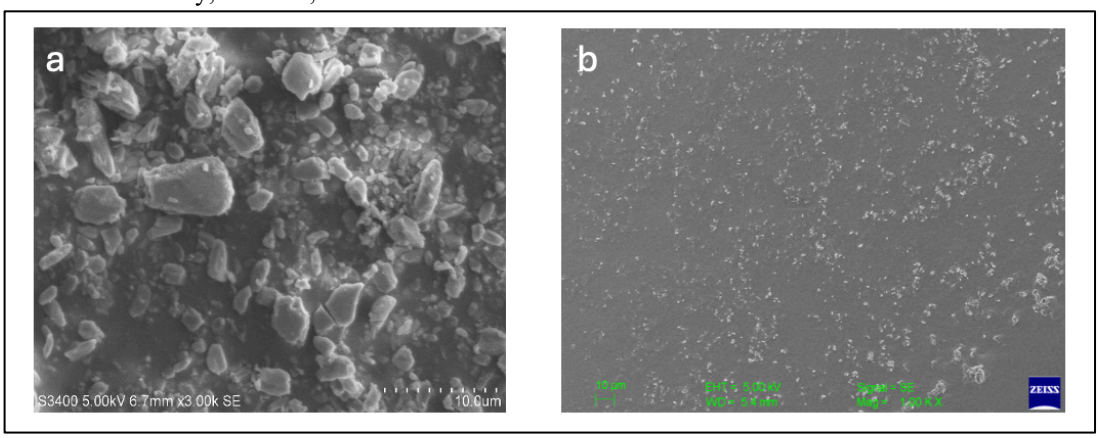

Figure 8 : a. FE SEM of TDF b. FE SEM of TDF NS

3.4.9 In vitro dissolution study

The comparative dissolution profiles of optimized NS, un-milled pure drug are presented in Figure 9. More than 40 % of drug was released within 15 minutes from NS as compared to only 5 % drug release from the pure drug. About six times more drug was released from NS indicating faster dissolution. The drug release pattern was similar even after 60 minutes as apparent from more than 90 % and about 20 % drug release from NS and pure drug respectively. After 2 hours, only 30 % of the pure drug was dissolved whereas complete dissolution was achieved in the case of NS. The results indicate that TDF NS exhibited better dissolution property than the pure TDF. The accelerated dissolution rate of TDF NS could be mainly due to the enhanced surface area rendered by nano-sized drug particles. These findings followed the Noyes–Whitney equation, suggesting that the drug dissolution rate is directly proportional to the surface area exposed to the dissolution medium. [20, 32]

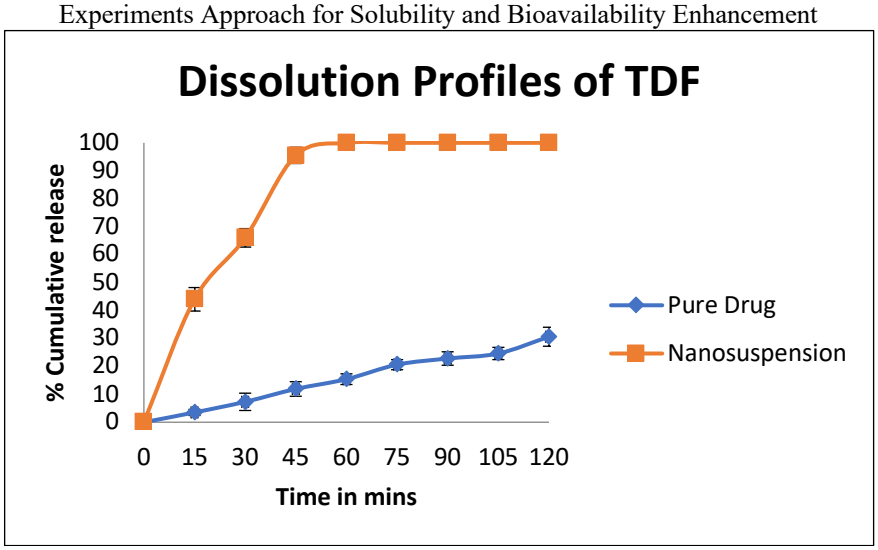

Figure 9: Comparative dissolution profiles of pure drug and NS of TDF

3.4.10 Stability studies

A stability study was performed on the NS stored at 40 ± 2 °C and 75 ± 5% relative humidity for three months to establish stability limits and guide storage recommendations. Particle size, PDI, and drug loading (%) were measured immediately after preparation and periodically throughout the three-month period. (Table 5). There was less than 5% change in the values of the data for particle size, PDI and % drug loading after 3 months. The formulation was considered stable since after 3 months a change from the initial assay of 5 % or more was not observed as recommended by ICH Q1A R2 stability guidelines.

Table 5 : Stability data of optimized TDF NS

Evaluation Parameters	Initial	1month	2 months	3 months	% Change
Particle size* (nm)	515.9±42	513±35	530.5±22	534.6±46	3.62
PDI*	0.539±0.034	0.535±0.06	0.537±0.024	0.548±0.021	1.67
Drug loading* (%)	70±1.2	69±1.5	69.5±0.7	69.4±2	0.86

***Mean±SD(n=3)**

3.4.11 In vivo pharmacokinetic studies

An in vivo study in rats was conducted to compare the pharmacokinetics of TDF NS and coarse drug suspension after oral administration. Cmax and AUC were evaluated to determine the rate and extent of absorption, assessing the effect of nanoparticulate formulation on oral bioavailability.[20] The results (Figure 10) display a 1.2-fold higher C_{max} (1479.16 ±189.50µg/ml) of NS when compared to pure suspension (1221.49 ± 375.67µg/ml). In addition, the systemic exposure level ($AUC_{0\text{-}48\ h}$) was much higher for NS(4927.34± 1700.31µg/ml*h) than for pure suspension (1944.57 ± 987.84µg/ml*h); the relative bioavailability was 2.5-fold for NS concerning that of pure TDF suspension. These results connote enhanced oral absorption of TDF in rats from NS formulation. The outcome of the in vivo study revealed improved bioavailability of TDF through NS formulation. The enhanced absorption is attributed to a greater dissolution rate, increased wettability and increased surface area by reduced particle size. [33] These findings also suggest the reduction of drug dose which is not only favourable economically but also desirable in minimizing the side effects associated with multiple dosage regimens.

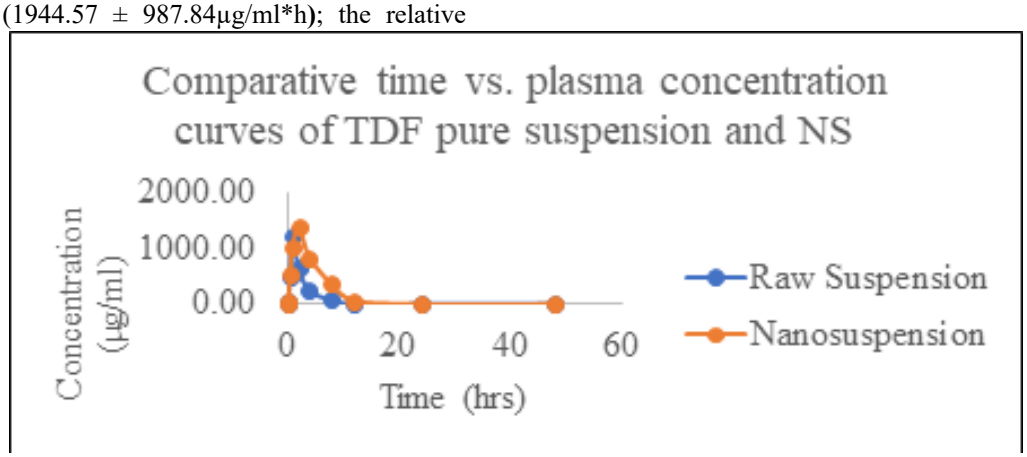

Figure 10: Comparative time vs plasma concentration curves of TDF oral pure suspension and NS

Alternative solubility enhancement approaches have also been reported for TDF. Amorphous solid dispersions [34] prepared by spray drying or hot melt extrusion typically yield very high apparent solubility

and can sustain supersaturation in gastrointestinal fluids; however, their long-term stability is limited by the inherent tendency of the amorphous drug to recrystallize under humid or thermal stress. Cyclodextrin inclusion complexes [35] have shown improved aqueous solubility and faster dissolution, but require high excipient loads and may not be cost-effective for high-dose formulations. Lipid-based systems, including self-nanoemulsifying drug delivery systems (SNEDDS) [6], produce rapid drug release and may promote lymphatic transport; nevertheless, their drug loading capacity is often restricted, and variability in absorption can arise due to food effects. Other nanocrystal preparation techniques, such as antisolvent precipitation coupled with sonication [36], yield particles of comparable dimensions to HSH NS, but solvent removal and scale-up challenges limit their applicability.

In comparison, the HSH-based NS offers a balanced profile by combining robust dissolution enhancement with high drug loading, relatively simple excipient composition, and favourable manufacturability. The improved oral bioavailability observed with TDF NS in preclinical evaluations further highlights their translational potential. Thus, while multiple formulation strategies exist to address TDF's solubility limitation, HSH NS emerges as a promising and scalable option that can bridge laboratory development and industrial production.

4. CONCLUSION

This study successfully developed and optimized a stable TDF NS using HSH coupled with BBD. The optimized formulation exhibited significantly enhanced solubility, faster dissolution, and a 2.4-fold increase in relative bioavailability compared with pure TDF. These outcomes establish NS technology as a scalable and robust platform to overcome solubility and bioavailability challenges of BCS Class II drugs. Clinically, such improvements in dissolution and absorption may translate into enhanced therapeutic efficacy, reduced interpatient variability, and potentially lower dosing requirements, thereby improving treatment outcomes and patient compliance in TDF therapy for erectile dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension. Future investigations focusing on NS solidification could further support the development of stable, patient-friendly solid dosage forms and expand the applicability of this strategy to other poorly soluble drug candidates.

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