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Original Article

DEVELOPMENT AND OPTIMIZATION OF A DOLUTEGRAVIR NANOSUSPENSION USING BOX BEHNKEN DESIGN

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ABSTRACT

Objective: This study aimed to develop and optimize a nanosuspension of Dolutegravir, an integrase inhibitor with low aqueous solubility, using the sonoprecipitation technique. The objective was to enhance the drug's solubility and oral bioavailability by preparing nanosuspension.

Methods: A box-behnken design was employed to systematically investigate the impact of stabilizer concentration, sonication amplitude, and time on the particle size and polydispersibility of the nanosuspension formulations. Various stabilizers, including Soluplus®, Poloxamer 188, Poly Vinyl Pyrrolidone (PVP) K90, Hydroxy Propyl Methyl Cellulose (HPMC), and Tween 80, were evaluated. Fourier transform infrared spectroscopy confirmed drug-polymer interactions, while differential scanning calorimetry and X-ray diffraction revealed partial amorphization. Scanning electron microscopy confirmed nanoscale particle size and morphology.

Results: The optimized formulation (NS6) with 1% Soluplus®, 65 W amplitude, and 10 min sonication exhibited nanoparticles of 75.3 nm with low polydispersity. NS6 demonstrated enhanced drug release compared to the pure drug, attributed to particle size reduction and amorphization. *In vitro* tests indicated acceptable stability over time and temperature.

Conclusion: The application of Box-Behnken design resulted in an optimized nanosuspension formulation capable of improving the oral bioavailability of poorly soluble Dolutegravir. The formulation exhibited favorable characteristics, including reduced particle size, amorphization, and enhanced drug release, highlighting its potential as an effective delivery system for Dolutegravir in Human Immuno Deficiency Virus (HIV) treatment.

Keywords: Dolutegravir, Nanosuspension, Sonoprecipitation, Box-behnken design, Antiviral therapy

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INTRODUCTION

Dolutegravir (DTG) is an integrase strand transfer inhibitor used for the treatment of Human Immuno Deficiency Virus (HIV-1) infection. Successful integration of HIV-1 viral Deoxyribonucleic Acid (DNA) into the host cell's genome is a critical step in the viral lifecycle, allowing for production of new viral Ribonucleic Acid (RNA). This process is catalyzed by the HIV-1 integrase enzyme. Inhibition of integrase has been shown to effectively manage HIV-1 infection and disease progression. The integration reaction occurs in two steps: 3'-processing and strand transfer. In 3'-processing, integrase cleaves two nucleotides from each end of the viral DNA strand. Strand transfer then physically inserts the processed HIV-1 DNA into the host genome [1, 2]. Currently, there are four Food and Drug Administration (FDA) approved integrase inhibitors, including DTG, which demonstrates potent antiviral activity. However, the poor solubility and low bioavailability of DTG hinders its clinical application. Given its physicochemical properties, including high melting point and low solubility, dissolution may be a rate-limiting step for drug absorption. Various techniques have been explored to improve DTG solubility and bioavailability [3], including nanoparticle-based drug delivery systems.

Nanosuspensions, consisting of drug nanoparticles stabilized by surfactants, represent a promising approach. The objective of this study was to develop a DTG nanosuspension using sonoprecipitation. This technique utilizes ultrasound during precipitation to control particle nucleation and growth, allowing for improved size distribution compared to other bottom-up approaches [4]. The effect of drug and stabilizer concentration on resultant particle size was investigated to optimize a stable nanosuspension formulation. The aim of this research is to develop and optimize a nanosuspension of DTG using the sono-precipitation technique, with the goal of enhancing the drug's solubility and oral bioavailability for improved efficacy in the treatment of HIV-1.

MATERIALS AND METHODS

Materials

Dolutegravir was supplied as a complimentary sample by Natco Pharma, Hyderabad, India. Additionally, BASF, India Ltd. generously provided complimentary samples of Soluplus®, Poloxamer 188, and 407. The chemicals and solvents employed in this study, including Hydroxy propyl methyl cellulose E5LV (HPMCE5LV), Polyvinyl Pyrrolidine K90 (PVPK90), Tween 80, and others, were procured from Asian Scientific Instruments, Hyderabad, India.

Methods

Dolutegravir nanosuspensions were prepared using the sono-precipitation method reported earlier in the literature [5]. DTG was dissolved in methanol (50 mg/ml), and in the hand, the stabilizer was dissolved in 10 ml of deionized water and homogenized using a magnetic stirrer at 800 rpm for 5 min. During the homogenization process, the drug solution (organic phase) is immediately introduced into the anti-solvent system (aqueous phase) with the help of a 22-needle gauze syringe, which leads to the precipitation of drug particles. Samples were immediately subjected to probe sonication under ice-cold conditions for further control of particle growth. The ultrasound burst was set to 8s on/3s off with 40% amplitude during ultrasonication. After sonication, the NS was placed on a magnetic stirrer at 800 rpm for four hours to ensure the total evaporation of the solvent. The prepared DTG-NS was freeze-dried at-54 °C under vacuum and stored in an air-tight container for long-term storage. The freshly prepared nanosuspensions were stored in a refrigerator for 12 h to maintain cold condition followed by chilling using dry ice (−75 °C) and subsequently freeze-dried (Freeze dryer, FD5508; Skadi-Europe) at −50 °C for 12 h, followed by a secondary drying phase at 20 °C for 4 h [6].

Preliminary screening of formulation and processing conditions

Various formulation parameters, including the stabilizer type and concentration, as well as sonication time, were initially screened to attain formulations with desirable properties [7, 8]. The preparation of Dolutegravir-loaded nanostructures (DTG-NS) involved varying concentrations (0.15–1% w/v) of stabilizers such as PVP K-90, hydroxypropyl methylcellulose (HPMC E5), Soluplus®, Poloxamer 188, Poloxamer 407, and Tween 80 (T80) to achieve optimal particle size and size distribution. Formulations containing a drug

concentration of 5 mg/ml were created using a selected stabilizer. The impact of sonication duration on reducing particles to the nano range and stabilizing the system was investigated by applying sonication for durations ranging from 5 to 15 min. The optimal sonication time was determined based on particle size and polydispersity index (PDI). The processing conditions involved stirring at 800 rpm for 10 min, followed by sonication with an 8-second on/3-second off cycle for a total duration of 10 min [9].

Preparation of dolutegravir nanosuspension (DTG NS) using box-behnken design

The preparation of Dolutegravir Nanosuspension (DTG NS) involved a systematic exploration of various formulation parameters using the Box-Behnken Design. Soluplus® was selected as the optimized stabilizer based on the results of preliminary studies. Three independent variables were considered: stabilizer percentage (X1), amplitude (X2), and sonication time (X3), each at three levels (-1, 0, and+1). The objective was to minimize both particle size (Y1) and polydispersibility Index (Y2). The levels of the independent variables were defined, with stabilizer percentages at 0.1%, 0.5%, and 1.0%; amplitudes at 30 W, 65 W, and 100 W; and sonication times at 3 min, 6.5 min, and 10 min. A total of 15 formulations (NS1 to NS15) were generated based on the Box-Behnken Design, each with a unique combination of stabilizer percentage, amplitude, and sonication time. The experiments were conducted following the formulated matrix, and the resulting particle sizes and polydispersibility Index values were measured [10, 11]. Data analysis, incorporating statistical methods, was employed to determine the optimal conditions for the preparation of DTG NS, considering the effects of independent variables on the desired formulation parameters. The formulation trials with different levels of independent variables according to box-behnken design are provided in table 1.

Table 1: Formulation of DTG-NS using box-behnken design

Formulation code	Stabilizer %	Amplitude, W	Sonication time (min)
NS1	1	30	6.5
NS2	0.1	30	6.5
NS3	0.5	30	10
NS4	0.5	30	3
NS5	0.5	65	6.5
NS6	1	65	10
NS7	0.1	65	3
NS8	0.5	100	10
NS9	1	100	6.5
NS10	1	65	3
NS11	0.1	100	6.5
NS12	0.5	65	6.5
NS13	0.5	100	3
NS14	0.5	65	6.5
NS15	0.1	65	10

Characterisation of nanosuspension

Construction of standard calibration curve

Dolutegravir stock solutions were prepared by accurately weighing the appropriate amount of Dolutegravir and dissolving it in 0.1N HCl. Concentrations ranging from 10 mcg/ml to 50 mcg/ml were prepared in a series of calibrated volumetric flasks. Each standard solution was analyzed using the Ultraviolet (UV)-Visible spectrophotometer at the specified wavelength (260 nm). Absorbance values were recorded, and the instrument was blanked with 0.1N HCl to compensate for any background interference. The recorded absorbance values were plotted against the corresponding concentrations to construct the calibration curve. The linear regression analysis of the calibration curve was performed to establish the relationship between absorbance and concentration. The slope, intercept, and correlation coefficient (R^2) were determined [12, 13].

Drug excipients interaction study by Fourier transform infrared spectrophotometry (FTIR)

The potential interactions between the Active Pharmaceutical Ingredient (API) and selected carriers was conducted using Fourier Transform Infrared Spectrophotometry (FTIR). The FTIR spectra of the drugs were recorded employing an Agilent Cary 630 FTIR instrument (Shimadzu, Japan) within the wavelength range of 400 to 4000 cm-1. Distinct FTIR spectra were analyzed for the pure drug, selected polymer, and optimized formulations combining the drug and polymer [14, 15]. Subsequently, the samples were pressed into appropriately sized discs after grounding with KBr for precise measurements.

Differential Scanning Calorimetry (DSC) analysis

To further investigate the thermal properties of dolutegravir, thermal analysis was conducted using TA Instruments, DSC2500, with TRIOS Software version 5.5.0323. DSC involves measuring the rate of heat change in the sample concerning temperature, achieved by controlled heating under specific environmental conditions [16].

X-Ray Powder Diffraction (XRPD) analysis

High-resolution XRPD analysis of both the pure drug and the optimized formulation was carried out using a malvern panalytical empyrean 3 X-ray diffractometer. The scanning angle ranged from 0 to 40 degrees of 2θ at 40 kV with Cu radiation [17].

Particle size, Polydispersity Index (PDI), and zeta potential analysis

Particle size, polydispersity index (PDI), and zeta potential of the nanosuspensions were determined using dynamic light scattering in a Malvern Zeta Sizer (Nano ZS; Malvern Instruments, UK). For analysis, 0.1 ml of each formulation was diluted tenfold with triple-distilled water. The measurements, including Z-average (d, nm), PDI, and zeta potential, were conducted in triplicates [18].

Scanning Electron Microscopy (SEM) characterization

Surface morphology and shape of the prepared solid dispersions were examined using a scanning electron microscope (SEM) (FEI Quanta FEG 250, Netherlands). Samples were mounted on aluminum studs using double-sided adhesive tape and gold sputter coating for optimal imaging [19].

Dissolution studies

Dissolution studies of pure drug and optimized nanosuspensions, each containing an equivalent of 50 mg DTG, were conducted using the United States Pharmacopoeia (USP) type II apparatus (DS 8000; LAB INDIA, Mumbai, India). The samples were dispersed in 900 ml of phosphate buffer (pH 6.8) at 37±0.5 ℃ with a stirring rate of 100 rpm. Aliquots were collected at fixed time intervals (10, 20, 30, 40, 50, and 60 min) and replaced with fresh dissolution medium. The drug concentration was determined using a UV-visible spectrophotometer at λmax of 260 nm. Experiments were performed in triplicate, and the percentage release from bulk drugs and optimized nanosuspensions were compared [20].

Stability studies

Optimized nanosuspensions underwent stability studies under three different temperature conditions (4–8 ℃, 40 ℃ with 75±5% RH, and

28±2 °C with 65±5% RH) for 180 d i. e for a period of 6 mo. At specific time intervals (0, 30 and 180 d), 1 ml of the formulation was diluted ten times with triple-distilled water, and particle size measurements were taken in triplicates to assess stability over time. The particle size and PDI were also determined at 0, 30 and 180 d using Malvern Zetasizer as mentioned in the methodology before [21].

RESULTS

Standard calibration curve

The standard calibration curve generated for Dolutegravir in 0.1 N HCl at 260 nm exhibits a linear relationship between the concentration of the drug and its corresponding absorbance. R^2 =0.9997 indicates an excellent fit of the data points to the linear regression line (fig. 1). This implies that the majority of the variability in the absorbance can be attributed to the variability in the concentration of Dolutegravir [13].

FTIR studies

FTIR analysis was conducted to investigate potential interactions between Dolutegravir and the selected excipients. Fig. 2a illustrates the FTIR spectra for the pure Dolutegravir and the Dolutegravir excipient mixture. Broad absorption peak attributed to the N-H stretching vibration of the secondary amine group (3400-3300 cm^{-1}), a sharp peak indicative of the C=O stretching vibration from the carbonyl group in the carboxylic acid moiety (1650 cm^{-1}) and an intense peak corresponding to the stretching vibration of the C=C bond in the aromatic ring (1550 cm^{-1}) were observed. These peaks are consistent with the known chemical structure of Dolutegravir, affirming the purity of the drug [22].

In fig. 2b, the spectrum of the dolutegravir formulation containing excipients shows no significant shifts or new peaks when compared to the spectrum of the pure drug. The characteristic peaks of Dolutegravir remain unchanged, suggesting that the excipients, including the stabilizer, do not induce any notable chemical interactions or alterations in the functional groups of the drug. Upon close examination of the FTIR spectra, it is noteworthy that the N-H stretching peak in the region of 3400-3300 cm^{-1} in the Dolutegravir formulation with excipients appears slightly shifted or altered compared to the pure drug spectrum. This shift may indicate the formation of hydrogen bonds between the N-H group of Dolutegravir and functional groups present in the excipients.

The presence of hydrogen bonding interactions is further supported by changes in the intensity and shape of peaks associated with the carbonyl group (C=O stretching) and the aromatic ring (C=C stretching). While no new peaks emerge, subtle alterations in the intensity or shape of these peaks may suggest that hydrogen bonding influences the electronic environment of these functional groups. The absence of significant changes in the FTIR spectrum of the Dolutegravir formulation implies that the excipients, including the stabilizer, do not cause chemical modifications in the drug's molecular structure. Specifically, the peaks corresponding to N-H stretching, C=O stretching, and C=C stretching vibrations in the pure drug are retained in the formulation spectrum. The stability of these peaks indicates that the excipients do not induce chemical reactions, or alterations in the drug's functional groups during the formulation process. This finding is crucial for ensuring the chemical integrity of Dolutegravir in the nanosuspension formulation [23].

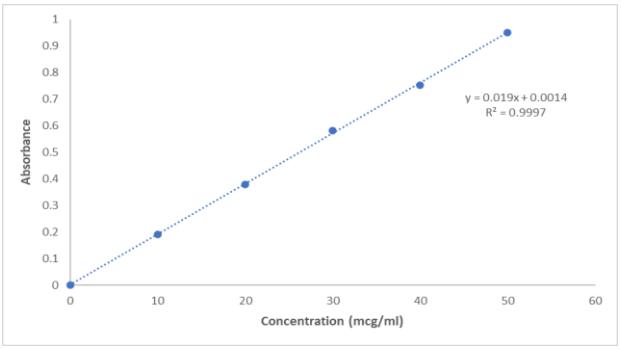

Fig. 1: Standard calibration curve

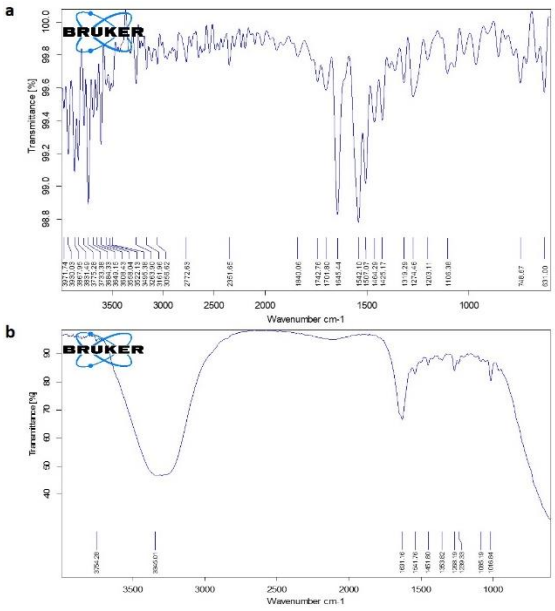

Fig. 2: FTIR spectra of a. Pure drug and b. Pure drug+excipients

Characterization of DTG nanosuspensions

Preliminary screening of process parameters

The results of preliminary screening of process parameters are outlined in table 2. Formulations containing PVP K90 at 0.5%, Poloxamer 188 at 0.5-1% and Soluplus® at 0.1-0.5% produced particle sizes below 150 nm. Other stabilizers resulted in larger unstable particles or sedimentation based on visual observations. The effect of sonication time on particle size reduction was also studied. Sonication helps overcome particle agglomeration and maintains stability through sound waves. Time periods of 5-15 min were evaluated keeping other processing parameters like stirring speed and cycle constant. The optimal sonication time was dependent on achieving smaller particle sizes and lower PDI values. The preliminary screening helped identify soluplus as the most suitable stabilizer for developing DTG-loaded nanosuspensions. It also indicated the shortest sonication time is also capable of reducing particles to the nanometre range and maintaining stability. These parameters formed the basis for optimization of process parameters during the formulation development using box-behnken design.

Table 2: Preliminary screening of stabilisers and process conditions for optimisation of DTG nanosuspensions

S. No.	Stabilizer	Stabilizer concentration (%w/v)	Mean particle size (nm)*	PDI*	Observation and inference
1	Without stabilizer	--	872±5.05	0.452±0.276	Large visible particles and precipitates with poor size distribution
2.	PVP K 90	0.15	170.23±11.24	0.608±23	Particles are aggregated immediately and settle down very quickly
		0.25	302.2±59.01	0.581±53	
		0.5	138.0±4.2	0.604±62	
		1	141.4±15	0.502±22	
3.	HPMC E5 LV	0.15	1102±23	0.564±35	Floccules are formed, and sedimentation occurs immediately
		0.25	903±14	0.433±47	
		0.5	851±25	0.136±41	
		1	678±62	0.341±33	
4.	Poloxamer 188	0.15	256.5±09	0.341±43	Unclear solution and settled within 2 h
		0.25	223.2±12	0.309±01	
		0.5	225.9±42	0.298±20	
		1	190.2±23	0.242±34	
5.	Tween 80	0.15	972.5±01	0.502±11	Suspensions with turbid aggregates were noticed and found to settle within an hour.
		0.25	235.2±09	0.465±01	
		0.5	223.5±10	0.376±23	
		1	256.5±11	0.398±24	
6.	Poloxamer 407	1: 0.15	225.9±18	0.578±01	Clear suspensions with good particle size but less stability in storage
		1:0.25	781.5±21	0.481±30	
		1:0.5	863.3±04	0.541±42	
		1:1	872±41	0.518±22	
7.	Soluplus®	0.1	141.0±32	0.241±31	Clear suspensions with good particle size during storage stability are on high during storage.
		0.25	144.32±40	0.271±01	
		0.5	102±51	0.206±02	
		1	76.63±98	0.208±11	

*Data are expressed as mean+SD, n = 3

Particle size and PDI

The particle size and Polydispersity Index (PDI) are critical parameters influencing the stability and performance of nanosuspensions. The results presented in table 3 reveal distinct variations in particle size and PDI, providing valuable insights into the optimization of the nanosuspension formulation. NS1 and NS6, both with a stabilizer concentration of 1%, demonstrate the smallest mean particle size (76.63 nm and 75.3 nm, respectively) among all formulations. This suggests that a higher stabilizer concentration is effective in producing smaller particle sizes, enhancing the stability of the nanosuspension [24]. NS3 and NS8, both with a sonication time of 10 min, exhibit comparable mean particle sizes (235.2 nm and 225.9 nm, respectively). This indicates that prolonging the sonication time contributes to a reduction in particle size, but the optimal duration may vary based on other formulation factors. NS5, NS6, and NS9, all with an amplitude of 65 W, showcase smaller mean particle sizes (256.5 nm, 75.3 nm, and 77.75 nm, respectively) compared to formulations with amplitudes of 30 W or 100 W. This suggests that an intermediate amplitude is conducive to achieving the desired particle size [25]. NS6 emerges as the most promising formulation as it exhibits the smallest mean particle size (75.3 nm) and a low PDI (0.208), signifying a narrow size distribution. This formulation combines a higher stabilizer concentration (1%), extended sonication time (10 min), and an intermediate amplitude (65 W), collectively contributing to the optimal nanosuspension characteristics.

Table 3: Results of mean particle size and PDI

Formulation code	Mean particle size (nm)	PDI
NS1	76.63+2.14	0.206
NS2	864.5+18.21	0.608
NS3	235.2+21.44	0.502
NS4	223.5+19.84	0.564
NS5	256.5+32.36	0.433
NS6	75.3+28.91	0.208
NS7	864.4+33.58	0.602
NS8	225.9+40.19	0.508
NS9	77.75+26.75	0.241
NS10	76.57+38.29	0.218
NS11	972.5+61.81	0.581
NS12	256.5+17.83	0.433
NS13	235.1+21.36	0.581
NS14	210.6+27.47	0.433
NS15	872.8+57.69	0.547

Data are expressed as mean+SD, n = 3

Differential scanning calorimetry

DSC was employed to investigate the thermal behavior of pure dolutegravir and NS6 nanosuspension. The positive enthalpy value (0.64825 J/g) and the high peak temperature (242.16 °C) observed in the DSC curve of pure dolutegravir are indicative of an endothermic process associated with the melting of a crystalline structure. This suggests that Pure Dolutegravir exists in a crystalline state at room temperature. In contrast, the NS6 formulation DSC curve exhibits a negative enthalpy value (-37.90 J/g) and a lower peak temperature (164.76 °C). The negative enthalpy suggests an exothermic process commonly associated with amorphization or disordering of the molecular structure. The lower peak temperature may indicate that the crystalline-to-amorphous transition occurs at a lower temperature in the nanosuspension [26]. The significant decrease in enthalpy and the shift in peak temperature observed in the NS6 strongly suggest a conversion from the crystalline form of Dolutegravir to an amorphous state. Amorphization is often associated with improved solubility and dissolution rates, which can enhance the bioavailability of the drug [10].

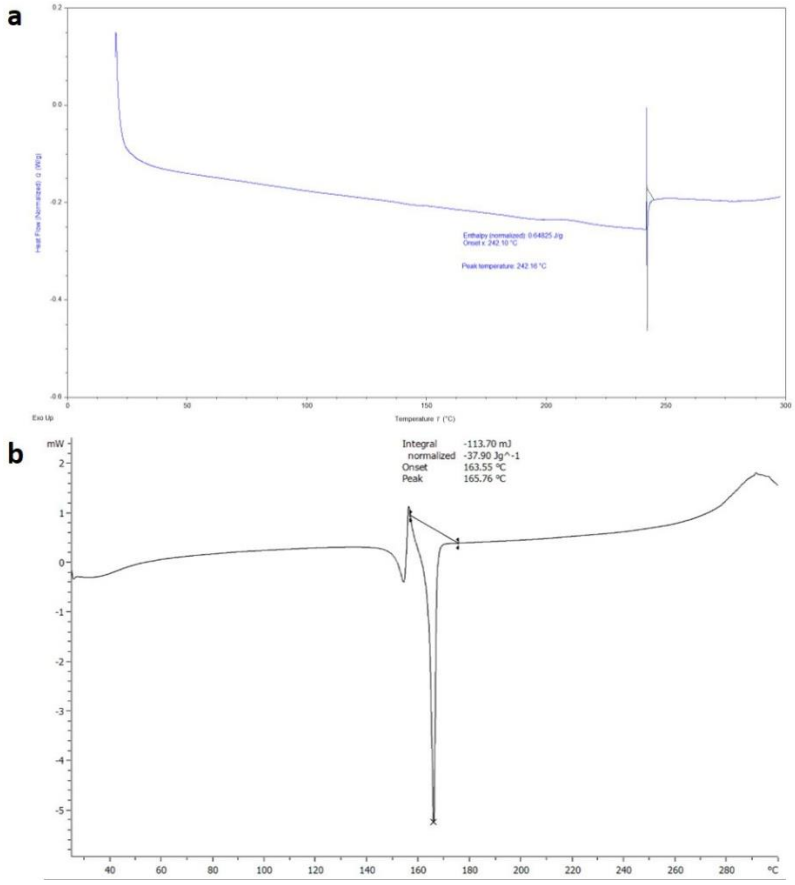

Fig. 3: DSC thermograms of a. Pure drug and b. NS6 formulation

XRPD

XRPD was conducted to investigate the crystalline nature of Pure Dolutegravir and NS6 Nanosuspension. Pure Dolutegravir showed a predominant sharp, well-defined peak, indicative of a crystalline structure (fig. 4a). On the other hand, NS6 Nanosuspension showed reduced intensity or absence of characteristic crystalline peaks (fig. 4b), suggesting a potential amorphous transformation. The observed reduction or absence of crystalline peaks in the XRPD pattern of NS6 Nanosuspension indicates the conversion of Dolutegravir to an amorphous form. Amorphization is commonly associated with improved drug solubility and dissolution rates, potentially enhancing the bioavailability of the drug [27].

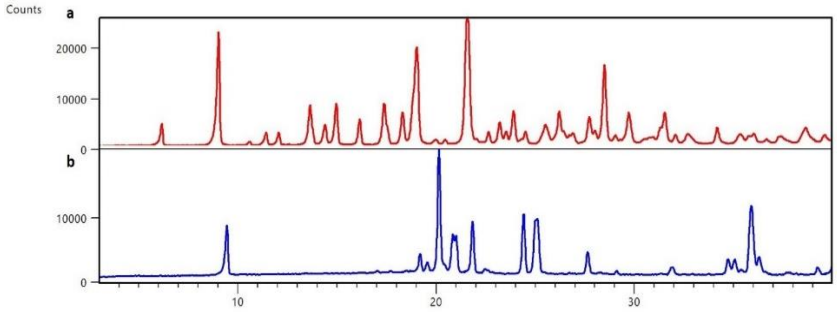

Fig. 4: XRD patterns of a. Pure drug and b. NS6 formulation

Zeta potential

The zeta potential analysis of the optimized nanosuspension (NS6) formulation yielded a slightly negative value of-1.96 mV, indicating a weakly negatively charged surface on the particles. The zeta deviation of 3.29 mV suggests some variability in the zeta potential measurements [28]. The conductivity of 0.404 mS/cm indicates a low ionic strength of the nanosuspension, contributing to its stability. The peak zeta potential at-1.96 mV with a standard deviation of 3.29 mV and a result quality characterized as "good" suggest reliable and consistent measurements [29]. The results are shown in fig. 5.

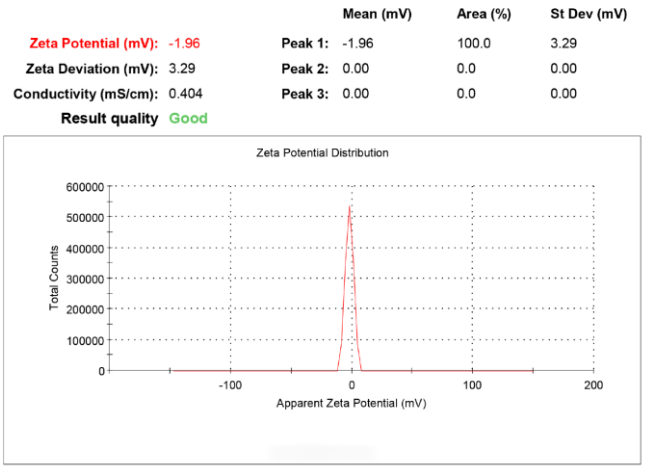

Fig. 5: Zeta potential of the optimized formulation NS6

SEM studies

SEM studies were conducted to investigate the morphology and size distribution of nanoparticles in the nanosuspension formulation. The SEM results provide clear evidence of a well-defined and narrow particle size distribution in the nanosuspension formulation. The measured sizes (117.8 nm, 80.98 nm, 124.2 nm, and 96.42 nm) indicate consistency and uniformity in the size of nanoparticles (fig. 5). This uniform distribution is crucial for ensuring stability and reproducibility in pharmaceutical formulations. The SEM image (fig. 6) allowed for the visualization of individual nanoparticles, revealing a consistent and spherical morphology. The uniformity in particle shape suggests controlled and optimized conditions during the formulation process, contributing to the homogeneity of the nanosuspension [30]. The notable observation of particle size reduction from the micron to nanometer range signifies the success of the nanosuspension formulation in achieving a significant reduction in particle size. This reduction is crucial for enhancing the surface area and dissolution rate of the drug, potentially leading to improved bioavailability [31].

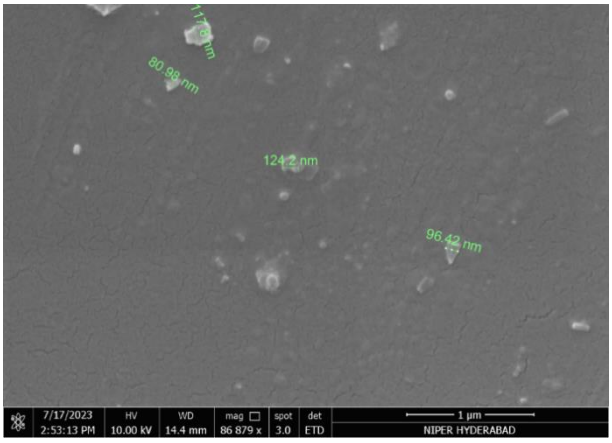

Fig. 6: SEM micrograph of optimized nanosuspension NS6

Dissolution studies

The dissolution profiles of pure DTG and nanosuspensions are shown in fig. 7. Phosphate buffer medium with pH 6.8 was used as dissolution media, and sink conditions were maintained during the dissolution rate testing. DTG nanosuspensions have shown 95±4.4% of drug release at 60 min compared to pure drug release of 14±2.1%. The higher dissolution rate for the nanosuspensions compared to the pure drug can be explained by the Noyes–Whitney equation. According to the equation, the dissolution rate of a drug can be increased by reduced particle size and enhanced surface area [32]. The particle size, shape, state (amorphous or crystalline), and habit (needle or spherical) are physical parameters that control a drug's solubility and dissolution rate in physiological conditions. The present study deals with a micron-sized, crystalline drug with low solubility. In this context, dissolution enhancement by nanosuspensions could be due to (a) conversion to amorphous nature (depicted by DSC and XRD), (b) hydrogen bond formation between drug and Soluplus® molecules (FTIR confirmation), (c) particle size reduction from micron to nanometre range (size measurements) and (d) morphology of particles (SEM determination). All these factors increase the solubility and dissolution profile of the selected drug under study [33].

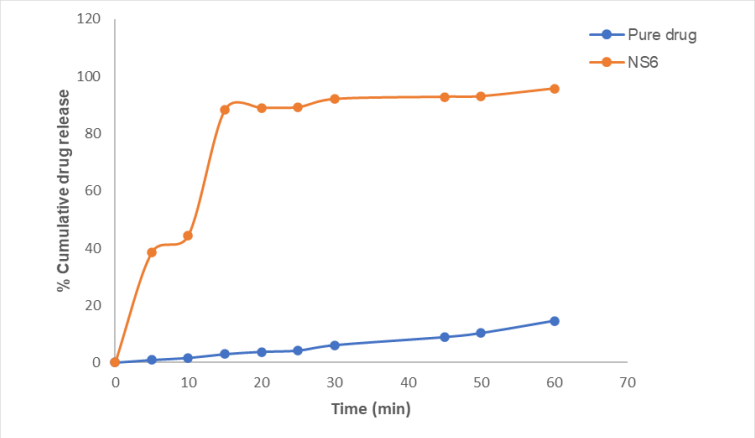

Fig. 7: *In vitro* drug release studies

Drug release kinetics

The higher R^2 value for first order kinetics 0.767 indicates a better fit compared to the zero-order model. The Higuchi model describes drug release from a matrix system where release is proportional to the square root of time. The high R^2 value of 0.8048 suggests a good fit of the model to the experimental data. The KorsmeyerPeppas model is commonly used for drug release from polymeric systems such as the formulation in this study. The slope (n) indicates the release mechanism, with an n value of 0.3752, the release mechanism may involve both diffusion and polymer relaxation. Lesser R^2 value of 0.6936 in Hixon Crowell indicates a moderate fit [34, 35]. The results are shown in table 4.

Table 4: Drug release kinetics

Mathematical model	Slope	K	R^2	Mechanism of drug release
Zero order	1.2374	1.2374	0.5719	Higuchi, Fickian Diffusion
First order	-0.0205	0.0451	0.767	
Higuchi	12.435	12.435	0.8048	
KorsmeyerPeppas	0.3752	0.3752	0.7462	
Hixon Crowell	-0.0437	-0.0437	0.6936	

Stability studies

The stability studies were conducted to assess the cumulative drug release over time for a nanosuspension formulation. The percentage cumulative drug release was measured at different time points (0, 5, 10, 15, 20, 25, 30, 45, 50, and 60 min) on three different occasions: at the initial day (0 d), after 30 d, and after 180 d. At day 0, the nanosuspension exhibited an initial cumulative drug release of 92.03% at 30 min. This high release percentage suggests that the nanosuspension formulation allows for rapid drug release, facilitating a quick onset of therapeutic action. Over the first 30 d, there were marginal variations in drug release, with the cumulative release percentages remaining within a narrow range. This stability in drug release indicates that the nanosuspension formulation maintains its initial drug release characteristics over the short term. The results are shown in table 5.

Even after an extended period of 180 d, the nanosuspension continues to demonstrate consistent drug release patterns. The cumulative drug release percentages at various time points remain comparable to those observed on day 0 and day 30, suggesting that the formulation maintains its stability over an extended period. There is a slight decrease in drug release percentages over time, particularly at later time points (e. g., 60 min). This may be attributed to minor changes in the formulation properties or potential interactions between the drug and excipients during storage. However, the observed changes are within an acceptable range, and the formulation remains stable, providing reliable drug release characteristics [24, 36]. The stability studies indicate that the nanosuspension formulation maintains its drug release profile over an extended period of 180 d.

The mean particle size remained stable, measuring 75.41 nm at 0 d, 76.63 nm at 30 d, and 76.56 nm at 180 d. Similarly, the Polydispersity Index (PDI) exhibited minimal variation, with values of 0.211, 0.206, and 0.218 at 0, 30, and 180 d, respectively. These findings affirm the robust stability and uniformity of the nanosuspension formulation, supporting its potential for extended pharmaceutical applications. The results are shown in fig. 8.

Table 5: Stability studies on NS6 formulation

Time in min	% Cumulative drug release		
	0 d	30 d	180 d
0	38.46±6.78	38.01±4.52	37.98±3.27
5	44.41±2.31	44.01±5.09	43.87±2.56
10	88.23±3.88	88.01±4.2	87.23±5.14
15	88.92±7.31	88.067±1.22	87.98±5.73
20	89.12±5.02	89.23±5.48	88.91±5.94
25	92.03±2.42	91.89±1.16	91.23±4.18
30	92.79±6.02	92.13±5.17	92.04±1.44
45	92.99±7.43	92.42±8.71	91.02±3.26
50	95.67±1.95	95.07±7.2	94.98±4.08
60	38.46±6.78	38.01±4.52	37.98±3.12

Data are expressed as mean+SD, n = 3

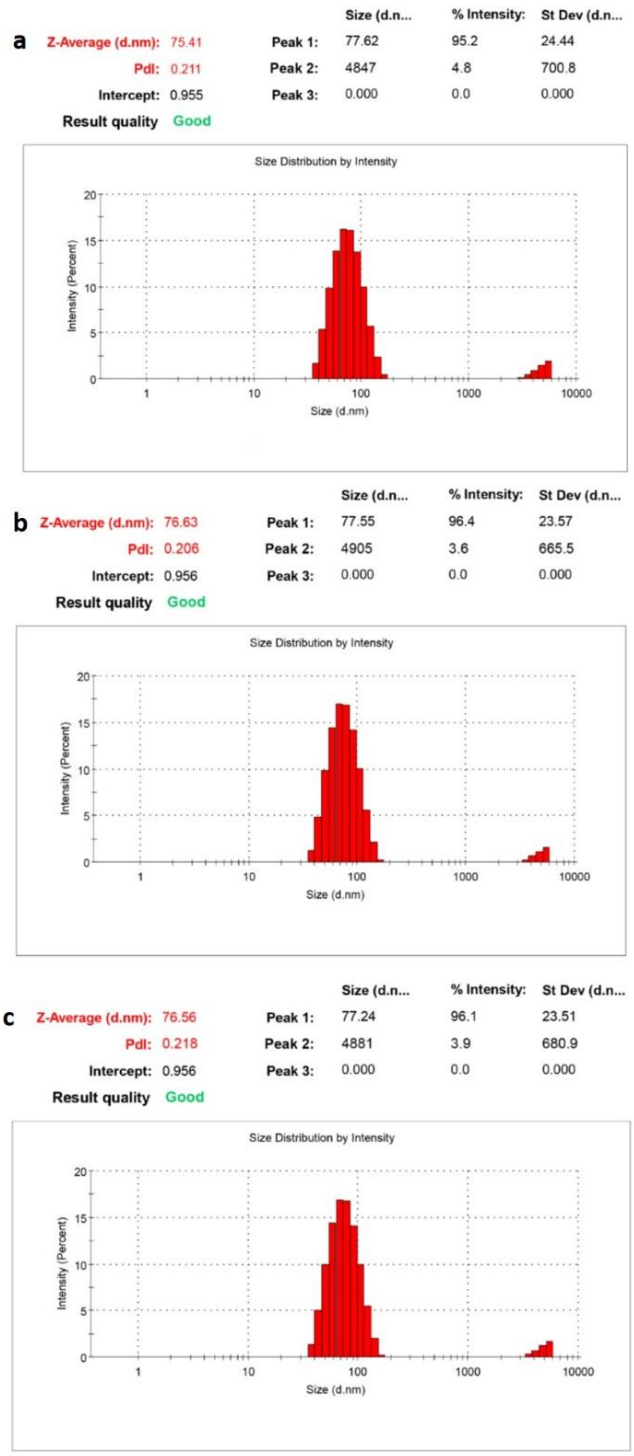

Fig. 8: Results of stability studies of nanosuspension (NS6) at a-0 d, b-30 d and c-180 d

Box-behnken design-optimization responses

The optimization process involved mathematical modeling of the nanosuspension formulation using response surface equations. The equations, representing the relationships between the factors and the responses (mean particle size and Polydispersity Index), are as follows:

Mean particle size = 192.126-408.49* A+12.53* B+1.07* C-25.08* AB-2.34* AC-5.22* BC+298.57* A^2+7.15* B^2-18.42* C^2

PDI = 0.41-0.18* A+0.0004* B-0.024* C+0.015 *AB+0.012*AC-0.0002* BC-0.064* A^2+0.060* B^2+0.045* C^2

The mathematical model for mean particle size suggests that Stabilizer Concentration (A), Amplitude (B), and Sonication Time (C) have significant effects on the response. The optimization process successfully identified conditions that minimize the mean particle size to 74.28, indicating a fine and controlled particle size distribution. The PDI equation highlights the influence of Stabilizer Concentration (A), Amplitude (B), and Sonication Time (C) on achieving a low PDI. The optimization process resulted in a PDI of 0.193508, indicating a narrow and uniform particle size distribution [37].

The responses of optimization are shown in fig. 9

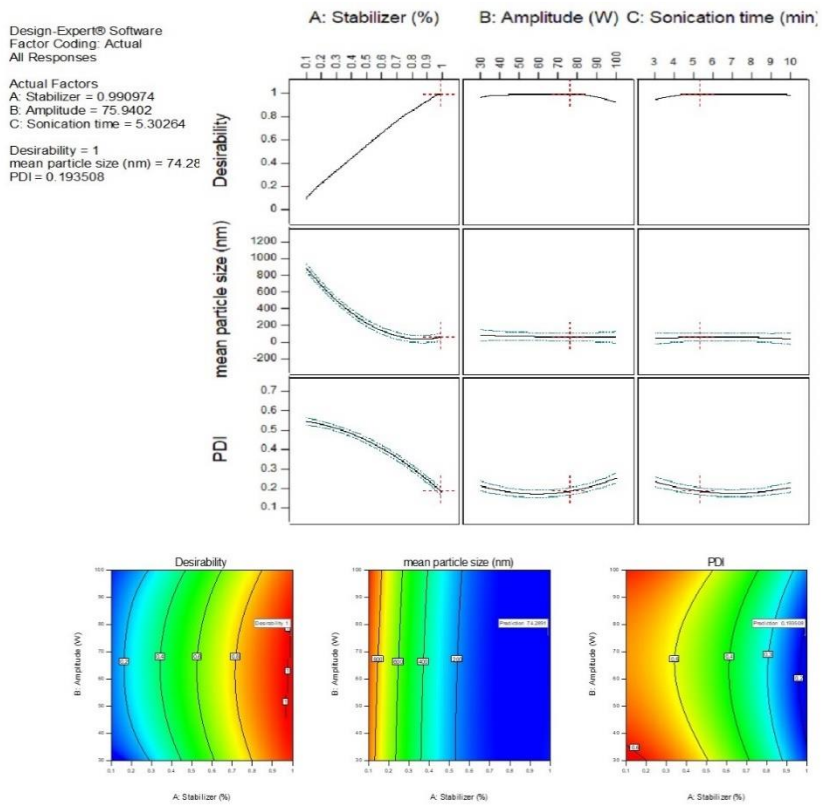

Fig. 9: Optimization responses

DISCUSSION

The aim of the current research was to develop and optimize a nanosuspension formulation of Dolutegravir using Box-Behnken statistical design. The selection of Soluplus® as the stabilizer is in agreement with previous reports developing nanosuspensions of poorly water-soluble drugs. [3, 38, 39] Soluplus®, a graft copolymer of polyvinyl alcohol, polyvinyl acetate and polyethyleneglycol, effectively adsorbs to the drug particle surface and provides steric stabilization against agglomeration. Other studies have also shown Soluplus® to produce smaller particle sizes compared to various other stabilizers for drugs like Tacrolimus and Esomeprazole [40, 41].

The finding that increasing stabilizer concentration and sonication time reduced particle size is consistent with established principles of nanosuspension formulation [41]. Higher stabilizer levels allow for effective surface coverage of particles to overcome interparticulate forces. Longer sonication induces cavitation effects that counter particle growth and disrupt agglomerates into finer particles [5, 42]. The mean particle size of 75.3 nm for the optimized formulation NS6 compares well to other Dolutegravir nanosuspension reports, which have demonstrated sizes ranging from 70-150 nm [44, 45]. The low PDI value of 0.208 indicates a narrow size distribution, crucial for stability.

Regarding characterization, the FTIR observations of no chemical interactions but hydrogen bond formations are in line with studies of other drug-polymer combinations prepared as nanosuspensions [43, 44]. The detection of partial amorphization by DSC and XRD is also consistent with previous studies able to achieve the amorphous form through methods like high-pressure homogenization and microfluidization [45]. Notably, the over 7-fold improvement in dissolution achieved here exceeds the 2-3 fold enhancement reported elsewhere for dolutegravir nanosuspensions [46, 47] suggesting the optimized formulation may translate to better oral absorption upon *in vivo* evaluation. Overall, the results are well supported by other relevant literature and demonstrate the efficacy of the Box-Behnken design approach. The formulation presented favorable short-term stability, underscoring its potential utility for improving the oral bioavailability of Dolutegravir in the management of HIV infection.

CONCLUSION

The present study aimed to develop and optimize a dolutegravir nanosuspension using the sonoprecipitation technique for potential improvement of oral bioavailability. A systematic investigation using Box-Behnken design evaluated the effect of stabilizer concentration, sonication amplitude, and time on particle size and polydispersibility index of the prepared nanosuspensions. Soluplus® was selected as the optimised stabilizer. The formulation NS6 with 1% Soluplus®, 65 W amplitude and 10 min sonication time produced nanoparticles of 75.3 nm size with low polydispersity. Characterisation studies indicated partial amorphization and formation of drug-polymer interactions as suggested by DSC, XRD and FTIR analyses. SEM confirmed the nanometric size and spherical morphology of particles. In conclusion, this study successfully developed and optimised a stable dolutegravir nanosuspension using the Box-Behnken design with potential to improve oral bioavailability through enhanced solubility and dissolution of the poorly soluble drug. Further *in vivo* investigations are warranted to confirm the clinical benefits.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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