RESEARCH PAPER

Quality by Design-Based Formulation and Evaluation of Dolutegravir Nanoparticles

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Abstract

Dolutegravir, a potent HIV integrase strand transfer inhibitor, exhibits limitations related to solubility and bioavailability, which may affect its therapeutic performance. The present study was aimed at the formulation and enhancement of Dolutegravir-loaded drug-enhancing polymeric nanoparticles entrapment and provide sustained drug release. Preformulation studies were carried out to characterize Dolutegravir with respect to its physicochemical properties, including organoleptic characteristics, melting point, solubility, UV-visible spectrophotometric analysis, and Fourier Transform Infrared (FT-IR) spectroscopy. The drug was found to be off-white, stable, soluble in methanol, dimethyl sulfoxide, and dichloromethane, and insoluble in water, with a λmax at 258 nm. FT-IR analyses verified the drug's structural soundness and compatibility with particular excipients.

Using ethyl cellulose as the polymer and polyvinyl alcohol as a stabilizing agent, dolutegravir nanoparticles were made via the emulsion solvent diffusion process. The impact of independent variables was investigated using a 3^2 complete factorial design. namely speed of rotation and polymer concentration, on entrapment effectiveness and particle size. Ready nanoparticles were evaluated for physical appearance, practical yield, entrapment efficiency, particle size, zeta potential, and in vitro drug release. The nanoparticles showed uniform appearance with practical yields ranging from 70.12% to 85.63%. It was discovered that entrapment efficiency ranged from 80.35% to 94.16%, increasing with higher polymer concentration and stirring speed.

Studies on in vitro release showed cumulative drug release and sustained drug release for up to 8 hours.ranging from 83.57% to 98.75%. Statistical analysis using Design-Expert software indicated that both rotational speed and ethyl cellulose concentration significantly influenced particle size and the effectiveness of trapping. The best possible formulation (F6) exhibited low particle size, high entrapment efficiency, sustained drug release, and satisfactory zeta potential (−20.3 mV), indicating good stability.

In conclusion, the study successfully developed and optimized Dolutegravir-loaded nanoparticles with improved entrapment efficiency and controlled drug release characteristics, suggesting their potential for enhanced oral delivery of Dolutegravir.

Keywords: Dolutegravir, Polymeric nanoparticles, Emulsion solvent diffusion method, Design of Experiments (DOE), 3^2 full factorial design, Entrapment efficiency, Nanoparticle drug delivery system, HIV therapy

How to cite this article: Patil LA, Sonar SD, Patil MD, Tare NV, Fegade TD, Borole TP. Quality by Design-Based Formulation and Evaluation of Dolutegravir Nanoparticles. Int J Drug Deliv Technol. 2026;16(19s): 829-845. DOI: 10.25258/ijddt.16.19s.97

Introduction

Despite great improvements in antiretroviral therapy (ART), HIV infection continues to be a major global public health concern. Long-term management of HIV requires lifelong administration of antiretroviral drugs, which often leads to issues such as poor patient compliance, frequent dosing, variable bioavailability, and drug-related adverse effects [1,2]. Hence, the design of advanced novel drug delivery systems which can augmenttherapeutic effectiveness with lowered dosing frequência and side effects has thus attracted substantialIn recent years,[3].

Dolutegravir is an integrase strand transfer inhibitor used for HIV-1 treatment.first-line antiretroviral drug because of its high potency, good resistance profile and more effectivesafety compared to earlier drugs[4,5]. However, Dolutegravir exhibits certain formulation challenges, including poor aqueous solubility and limited oral bioavailability, which can affect its therapeutic performance [6]. Conventional dosage forms may not provide sustained drug release, leading to fluctuations in plasma drug concentrations and reduced treatment adherence [7]. Hence, To get over these restrictions, innovative delivery methods must be developed.

Nanoparticle-mediated drug delivery systems represent a powerful strategy to enhance thesolubility and stability improvement, as well as the issue of bioavailability and controlled release, of poorly soluble drugs[8,9]. Particularly, polymeric nanoparticles provide benefits including improved drug loading, regulated and prolonged drug release, protection against drug degradation, and better therapeutic results.[10]. Ethyl cellulose, a widely used biocompatible and hydrophobic polymer, has shown excellent potential in sustaining drug release, while polyvinyl alcohol serves as an effective stabilizing agent during nanoparticle preparation [11,12]. Preformulation studies are vital to pharmaceutical development because they provide vital details about the physicochemical characteristics of the therapeutic material, including stability, compatibility with excipients, solubility, and melting point.[13]. These studies help in selecting suitable excipients and formulation techniques, assuring the creation of a dosage form that is both stable and efficient. Additionally, drug–excipient compatibility studies are essential to prevent any physical or chemical interactions that could compromise the quality, safety, or efficacy of the formulation [14].

The emulsion solvent diffusion method is a widely employed technique for the preparation of polymeric nanoparticles due to its simplicity, reproducibility, and ability to produce particles with controlled size and high drug entrapment efficiency [15]. Furthermore, application of factorial design and statistical optimization techniques allows systematic evaluation of formulation variables and their influence on critical quality attributes such as particle size and entrapment efficiency, thereby reducing experimental trials and improving formulation robustness [16].

The goal of the current study is to create and improve Dolutegravir-loaded polymeric nanoparticles using the emulsion solvent diffusion method.The study focuses on comprehensive preformulation evaluation of Dolutegravir, creation of nanoparticles with polyvinyl alcohol and ethyl cellulose, optimization using a full factorial design in three dimensions. In order to create a stable and efficient controlled drug delivery system for better HIV infection treatment, the produced nanoparticles were assessed for physicochemical properties, drug entrapment efficiency, in vitro drug release, particle size, and zeta potential.

Materials and Methods

Materials

A free sample of dolutegravir was acquired from a Mylan pharmaceutical company. Ethyl cellulose was used as the polymer, and polyvinyl alcohol (PVA) was used as a stabilizing agent. Dichloromethane (DCM) was used as an organic solvent, while methanol was used for analytical purposes. Every chemical and reagent used in the investigation was of analytical quality and was used exactly as supplied. Distilled water was used throughout the experimental work.

Preformulation Studies

To assess Dolutegravir's physicochemical characteristics and make sure it was appropriate for nanoparticle formulation, preformulation tests were conducted.

Drug Characterization

Dolutegravir's organoleptic characteristics, including color, smell, and appearance, were assessed visually. A tiny amount of the medication was checked for color and appearance in a well lit room. while odour was assessed by gentle smelling.

Determination of Melting Point

The open capillary method was used to measure the melting point of dolutegravir. A capillary tube with one end capped was filled with a tiny quantity of the medication and put in a melting point device. To evaluate purity, the temperature range at which the medication melted was noted and compared with published values.

Solubility Studies

Dolutegravir's solubility was investigated in a number of solvents, such as methanol,dimethyl sulfoxide, dichloromethane, and water. Approximately 20 mg of drug was added to 10 ML of each solvent were placed in different test tubes and sonicated for 10 min. The samples

were visually observed for complete dissolution or presence of undissolved particles.

UV–Visible Spectrophotometric Analysis

Dolutegravir was analyzed using a Jasco V-550 UV–visible spectrophotometer.Spectrophotometer for ultraviolet light. As the solvent, methanol was employed. 10 mg of dolutegravir were dissolved in 10 mL of methanol to create a stock solution (1000 µg/mL). From this stock solution, appropriate dilutions were made to determine the maximum absorption wavelength (λmax). The λmax of Dolutegravir was found at 258 nm.

Preparation of Calibration Curve

To create a calibration curve, the stock solution was diluted to yield concentrations between 5 and 25 µg/mL. Each dilution's absorbance was measured at 258 nm with methanol serving as a blank. Plotting the calibration curve between absorbance and concentration allowed for the establishment of linearity.

Fourier Transform Infrared (FT-IR) Spectroscopy

Dolutegravir's distinctive functional groups were identified and drug–excipient compatibility was evaluated using FT-IR spectroscopy. A Shimadzu IRAffinity-1 FT-IR spectrometer was used to record the FT-IR spectra of the pure drug and physical mixes containing excipients utilizing the KBr pellet method across a range of 400–4000 cm^{-1}.

Drug–Excipient Compatibility Study

Studies on the compatibility of drugs and excipients were conducted by physically mixing Dolutegravir with selected excipients in a 1:1 ratio. The mixtures were passed through a sieve, filled into glass vials, then kept for a month at 40 ± 2°C and 75 ± 5% relative humidity in a stability chamber. Samples were examined for physical alterations visually and examined using FT-IR spectroscopy to detect any possible interactions.

Preparation of Dolutegravir-Loaded Nanoparticles

Using the emulsion solvent diffusion approach, nanoparticles were created. In 20 milliliters of dichloromethane, ethyl cellulose and dolutegravir were dissolved to create the dispersion phase. Using a magnetic stirrer, 200 milligrams of polyvinyl alcohol were dissolved in 100 milliliters of distilled water to create the continuous phase.

Dropwise additions of the dispersed phase were made to the continuous phase while it was continuously stirred at fixed rotation rates. In order to promote solvent diffusion and nanoparticle production, stirring was maintained for four hours. The resultant nanoparticles were filtered, dried for 24 hours at 40°C in a hot air oven, and then stored in a vacuum desiccator to eliminate any remaining solvent.

Experimental Design

The impact of independent factors on nanoparticle properties was investigated using a 3^2 complete factorial design. The concentration of ethyl cellulose (X_2) and rotational speed (X_1) were chosen as independent variables. Particle size and entrapment efficiency were the dependent variables that were assessed. Statistical analysis and experimental design were done using Design-Expert® software.

Design of Experiments (DOE)

The impact of formulation and process variables on the crucial qualitative features of Dolutegravir-loaded polymeric nanoparticles was methodically investigated using a 3^2 complete factorial design. With the fewest possible experimental runs, the design was used to assess the main effects and interaction effects of two independent variables on particular dependent responses.

Evaluation of Nanoparticles

Physical Evaluation

Prepared nanoparticles were evaluated for colour, odour, and appearance by visual inspection.

Practical Yield

The theoretical weight of the medication and polymer utilized during formulation was compared with the actual weight of dried nanoparticles to determine the practical yield of nanoparticles. **Entrapment Efficiency**

An accurately weighed quantity of nanoparticles equal to 10 mg of dolutegravir was dissolved in methanol to measure entrapment effectiveness. The mixture was filtered, appropriately diluted, and subjected to spectrophotometric analysis at 258 nm. By dividing the actual drug content by the theoretical drug content, entrapment efficiency was determined.

In-Vitro Drug Release Study

The dialysis bag diffusion method was used for in vitro drug release investigations.

Dialysis bags containing 100 mg of nanoparticles were submerged in phosphate buffer (pH 6.8). At 100 rpm, the system was continuously stirred. At prearranged intervals, samples were taken out, replaced with new buffer, and subjected to spectrophotometric analysis at 258 nm.

Size of Particles

Particle Size Analysis

Using a Malvern Zetasizer and the Dynamic Light Scattering (DLS) method, the mean particle size and size distribution of nanoparticles were ascertained. Before analysis, samples were spread out in distilled water.

Zeta Potential Measurement

A Malvern Zetasizer was used to analyze the improved nanoparticle formulation's zeta potential in order to assess surface charge and stability. The dispersion medium was distilled water. **Statistical Analysis and Optimization**

Design-Expert® software was used to analyze the collected experimental data. The importance of the formulation variables was assessed using analysis of variance (ANOVA).In order to optimize the nanoparticle formulation based on smallest particle size and maximum entrapment performance, mathematical models and response surface plots were created.

1. Preformulation study:

Preformulation studies are a crucial phase in pharmaceutical development, where a drug substance's physical and chemical characteristics are analyzed before formulating it into a dosage form. This stage involves comprehensive investigation and characterization of the drug's inherent properties, including its solubility, stability, compatibility with excipients, particle size, and polymorphic forms. Understanding these characteristics helps in selecting the most suitable formulation approach and aids in predicting the drug's behavior during the manufacturing process and after administration. Preformulation studies lay the foundation for designing an effective and stable drug formulation, ensuring its safety, efficacy, and manufacturability.

A. Drug Characterization

i. Color: A little quantity of dolutegravir was placed on butter paper and inspected in a well- lit environment.

ii. Odor: To detect the odor, a tiny sample of dolutegravir was smelled.

iii. Appearance: Dolutegravir was pinched between two fingers, and the drug's appearance was noted.

B. Determination of melting point:

Melting point is the first indication of purity of the sample. Melting point of Dolutegravir was performed by open capillary method. Dolutegravir was taken in a glass capillary whose one end was sealed by flame. The capillary was then placed inside the melting point apparatus and melting point was noted.

C. Solubility study:

Dolutegravir's solubility was assessed in the several solvents listed in Table 1. Ten milliliters of the necessary solvent were introduced to a test tube along with twenty milligrams of dolutegravir. After 10 minutes of sonication, the mixture was examined to see if any particles remained.

Sr.no	Solvent
1.	Methanol
2.	Dimethyl sulfoxide
3.	Dichloromethane
4.	Water

Table 1: Solvents used for solubility study

D. UV-visible spectrophotometric analysis:

UV analysis: It was possible to obtain the Dolutegravir UV spectrum. Spectra Manager Software and a Japanese V 550 Spectrophotometer from Jasco Corporation were utilized for the analysis.

After giving the glassware, a thorough rinse with double-distilled water, it was dried.

Materials & Reagents: Methanol was used as a solvent to make dilutions, and all reagents were of analytical quality.

Method: 1000 µg/ml was produced by dissolving 10 milligrams of dolutegravir in 10 ml of solvent (methanol). A 20 µg/ml sample was obtained by diluting 0.2 ml of the produced solution with up to 10 ml of methanol, and spectra were recorded.

Calibration Curve preparation:

Stock solution: 1000µg/ml was created by dissolving 10 milligrams of dolutegravir in 10 ml of solvent (methanol).

Solution A: To make 100 µg/ml, 1 milliliter of the sample was taken out of the stock solution and diluted with 10 milliliters of methanol.

Absorbances were measured at 258 nm after 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.5 ml of solution A were taken out and diluted up to 10 ml with solvent (methanol) to create 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm.

E. FT-IR of Dolutegravir:

Dolutegravir's infrared spectrum was captured using the Shimadzu IRAffinity-1. Potassium bromide (KBr) was used as a blank to record the spectrum at a resolution of 4 cm over a range of 400–4000 cm. The principal peaks of the infrared spectrum described in the monograph were compared to the peaks in the Dolutegravir spectrum.

2. Drug excipient compatibility study:

Drug excipient compatibility studies represent an important phase in drug development. Before a drug substance is formulated into a desired dosage form, there is need for the formulator to fully consider the chemical structure of the drug substance, type of delivery system required and the proposed manufacturing process. Drug substances are usually combined with the excipients which serve different and specialized purpose. Excipients are pharmacologically inert, but given the right conditions they can undergo chemical reactions and physical interactions with drug molecules under favorable environmental conditions. Compatibility test on drug excipient have been used to approve or reject excipients for use in pharmaceutical formulation. The API alone and with individual excipients were taken in different ratios and mixed well. Passed through sieve, the blend was filled into the glass vials and kept in stability chamber at 40± 2°C/75 ± 5%RH.

Sr. No.	Sample	Ratio
1	Dolutegravir: Polyvinyl pyrrolidone	1:1
2	Dolutegravir: Ethyl cellulose	1:1
3	Dolutegravir: Dichloromethane	1:1

Table 2: Drug – Excipient compatibility study ratio

3. Preparation of nanoparticles by Emulsion solvent diffusion method:

1. The emulsion solvent diffusion method of producing nano sponges requires adjusting the ethyl cellulose ratio and rotational speed. Separate preparations are made for the dispersed phase and the continuous phase.
2. Ethyl cellulose and dolutegravir are dissolved in 20 milliliters of dichloromethane to create the dispersed phase.
3. A magnetic stirrer is used to dissolve 200 mg of polyvinyl alcohol in 100 ml of water (continuous phase).
4. The dispersed phase was then gradually introduced to the continuous phase. A magnetic stirrer is used to continually swirl the slurry for four hours at varying RPM according to formulation batches.
5. The resulting nanoparticles were filtered out and baked in an oven at 40°C for roughly a day.
6. After that, the nanoparticles were stored in vacuum desiccators to eliminate any remaining solvent before being used again.

4. Factorial Design model:

32 full factorial designs were used to create stable nanoparticles, and the results were satisfactory in terms of how changing the concentrations of variables like rotation speed (X1) and polymer concentration (Ethyl cellulose) (X2) affected responses like particle size and entrapment efficiency. The two elements' levels were chosen based on research done prior to putting the experimental design into practice. The experimental runs, their factor combinations, and the conversion of the coded levels to the study's experimental units are all summarized in the table.

Factor	Levels		
Independent variables	**Lower level**	**Medium level**	**Higher level**
Speed of rotation	1400	1600	1800
Drug: Ethyl cellulose ratio	1:1	1:1.5	1:2
Responses			
Particle size (nm)	Lowest		
Entrapment efficiency (%)	Highest		

Table 3: Factorial design for Nanoparticles

Sr. no.	Ingredients/ Parameters	Batches (Quantity)								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	Dolutegravir (mg)	100	100	100	100	100	100	100	100	100
2.	Polyvinyl alcohol (mg)	200	200	200	200	200	200	200	200	200
3.	Ethyl cellulose (mg)	100	100	100	150	150	150	200	200	200
4.	Speed of rotation (RPM)	14000	16000	18000	14000	16000	18000	14000	16000	18000
5.	Dichloromethane (ml)	20	20	20	20	20	20	20	20	20
6.	Distilled water (ml)	100	100	100	100	100	100	100	100	100

Table 4: Formulation strategy

5. Evaluation procedures:

1. **Physical examination:**

   Color: A small number of nanoparticles were placed on a glass slide and studied under bright light.

   Odor: To detect the odor, a sufficient number of nanoparticles were smelled.
   The quantity of nanoparticles was measured between two fingers, and their appearance was observed.

2. **Practical yield:**

   The weight of the dried final product in relation to the beginning weight of the medicine and polymer used to create the nanoparticles was used to calculate the practical yield of nanoparticles. The practical yield was calculated using the following formula.

$$\text{Practical yield} = \frac{\text{Theoretical weight of (Drug + Polymer)}}{\text{Practical weight of nanoparticles}} \text{ X 100}$$

3. **Entrapment efficiency:**

   To calculate the entrapment efficiency accurately weighed quantity of nanoparticles (10 mg equivalent of Dolutegravir) with 5 ml of methanol in a volumetric flask was shaken for 1 min using vortex mixer. The volume was made up to 10 ml with methanol. Then the solution was filtered, diluted and the concentration of Dolutegravir was determined spectrophotometrically at 258 nm.

$$\text{Entrapment efficiency} = \frac{\text{Actual drug content in nanoparticles}}{\text{Theoretical drug content}} \text{ X 100}$$

4. **In vitro release study:**

   Drug release was determined by dialysis method. 100 mg of each formulation was poured into dialysis bags and put into 25 ml phosphate buffer (pH 6.8 pH) and stirred (100 rpm, room temperature). At predetermined time intervals, 1 ml of aliquot was taken and then substituted by fresh phosphate buffer. Finally, suitable dilution was made with methanol and the amounts of released Dolutegravir in phosphate

buffer were measured by spectrophotometer at 258 nm. Aliquots withdrawn were assayed at each time interval for the drug released at λ max of 258 nm using UV-Visible spectrophotometer by keeping methanol as blank and the amount of released drug was estimated by the standard curve.

5. Particle size determination:

Using a Malvern zeta sizer at 25°C, the Dynamic Light Scattering technique was used to calculate the average mean diameter and size distribution of loaded nanoparticles. To achieve the appropriate light scattering intensity for dolutegravir nanoparticles, the dried nanoparticles were distributed in water.

6. Determination of zeta potential:

Surface charge is measured by zeta potential. Zeta sizers (Malvern Instrument) with zeta cells, polycarbonate cells with gold-plated electrodes, and water as a sample preparation medium can be used to measure the surface charge (electrophoretic mobility) of nanoparticles. It is crucial for characterizing the nanoparticles' stability.

1.Preformulation study:

A. Drug Characterization:

For the obtained drug samples, drug characterization criteria like color, odor, and appearance were examined; the findings are displayed in table 1.

Sr.no	Solvent	Observation
1.	Methanol	Soluble
2.	Dimethyl sulfoxide	Soluble
3.	Dichloromethane	Soluble
4.	Water	Insoluble

Colour	Off-White
Odour	Characteristic
Appearance	Fine powder

Table 1: Drug characterization parameters

B. Determination of melting point:

Dolutegravir's melting point was determined to be between 188 and 190 ^{0}C, which is consistent with its claimed melting point.

C. Solubility study:

Dolutegravir's solubility was investigated utilizing several solvent systems in accordance with the literature. Table 2 displayed the solubility data.

Table 2: Results for solubility study

D. UV-visible spectrophotometric analysis:

The UV-visible spectrophotometric analysis was carried out by using Jasco Corporation, Japan V 550 Spectrophotometer and spectra manager software was used for analysis. Methanol was used as solvent system for determination of λ_{max}. 20 µg/ml of Dolutegravir sample was used and λ_{max} was found as 258 nm. The spectra for results were expressed in figure 1 and 2.

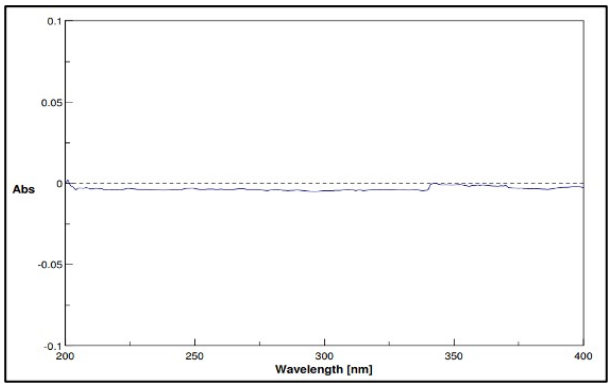


Figure 1: Blank in Methanol

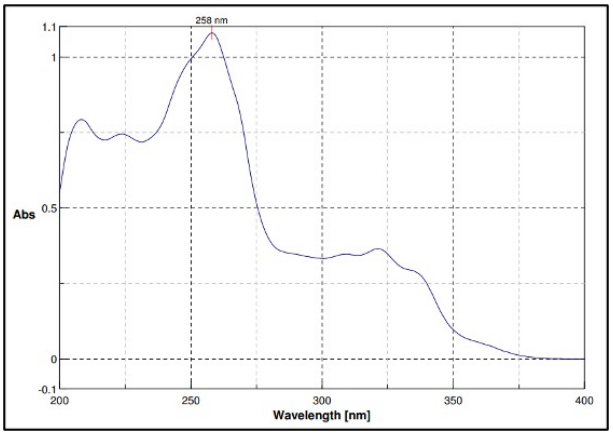


Figure 2: 20 PPM solution of Dolutegravir in Methanol

Preparation of Calibration curve for Dolutegravir in methanol:

Dolutegravir's calibration curve was created by measuring the absorbance of various methanol concentrations at 258 nm. Table 3 and Figure 3 displayed the calibration curve that was acquired.

Sr.no.	Concentration (ppm)	Absorbance
1.	5	0.2335
2.	10	0.5158
3.	15	0.7824
4.	20	1.0987
5.	25	1.2488

Table 3: Calibration curve for Dolutegravir

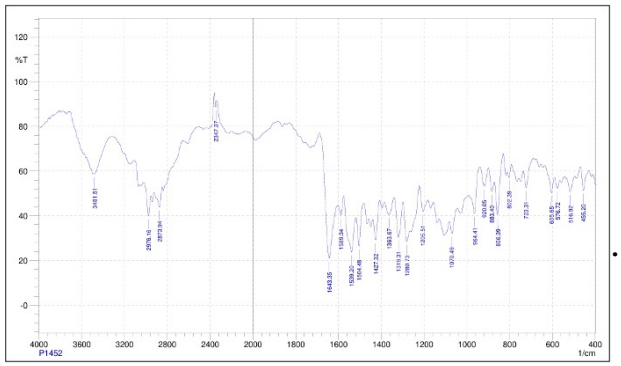


Figure 3: Calibration curve for Dolutegravir

In the concentration range of 5-25 µg/ml, the calibration curves were linear and followed Beer-Lambert's law. The data's excellent linearity was indicated by the correlation coefficient values of 0.9903.

E. FT-IR of Dolutegravir:

The IR spectrum of Dolutegravir was recorded by using FTIR spectrometer. IR spectra was shown in figure 4. Characteristic functional groups were observed in FTIR spectrum as shown in table 4.

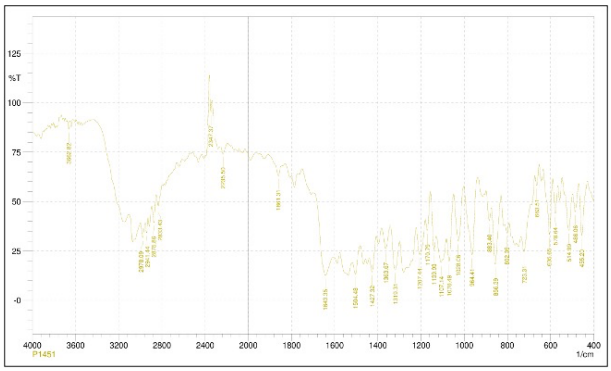


Figure 4: IR of Dolutegravir

Functional group	Observed Frequency	Reported Frequency
O-H Stretching (Alcohol)	3662.82	3700-3584
N-H Stretching (Amine salt)	2978.09	3000-2800
C=C Stretching (Conjugated alkene)	1643.35	1650-1600
O-H bending (Carboxylic acid)	1427.32	1440-1395
C-N Stretching (Aromatic amine)	1319.3	1342-1266
C-F Stretching (Floro compound)	1107.14	1400-1000

Table 4: IR frequencies of Dolutegravir functional group

. Drug excipient compatibility study:

The FTIR Spectra of Dolutegravir in pure form and their physical mixture was observed, the result showed that there was no interaction between drug, polymer and excipients. IR spectra for compatibility study were shown in figure 5, 6, 7 and their respective functional group detection data were shown in 5.

Figure 5: Compatibility IR for Dolutegravir: Polyvinyl alcohol

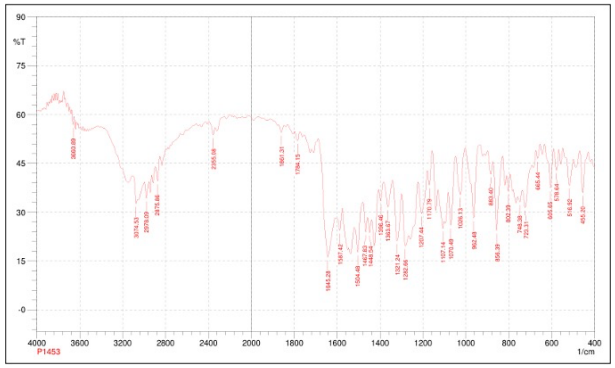


Figure 6: Compatibility IR for Dolutegravir: Ethyl cellulose

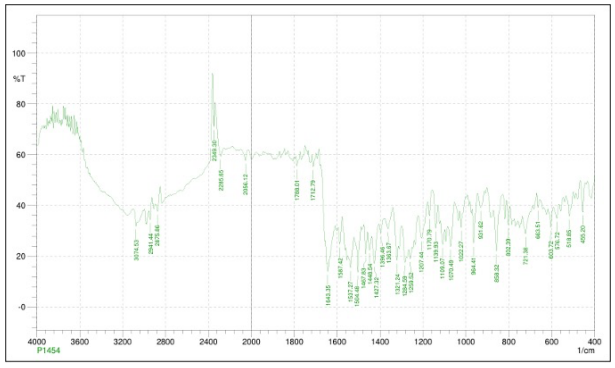


Figure 7: Compatibility IR for Dolutegravir: Dichloromethane

Ingredient	Initial observation	Condition
		40°C/75% RH (Accelerated)
		1 month
Dolutegravir	White	NCC
Dolutegravir : Polyvinyl alcohol	White	NCC
Dolutegravir : Ethyl cellulose	White	NCC
Dolutegravir : Dichloromethane	ransparent	NCC

Table 6: Drug excipient compatibility

Note- RH (relative humidity) and NCC (no conformational change) in physical appearance from the original description.The data above shows that all of the excipients utilized in the formulation and development of the dolutegravir combination were stable.

3. Formulation of Dolutegravir loaded Nanoparticles:

Sr.no.	Ingredients	Role
1.	Dolutegravir	HIV integrase inhibitor
2.	Polyvinyl alcohol	Stabilizing agent
3.	Ethyl cellulose	Polymer
4.	Dichloromethane	Solvent for polymer
5.	Distilled water	Dispersion medium

Table 7: Formulation ingredients and its roles

Formulation strategy:

Sr. no.	Ingredients/ Parameters	Batches								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	Dolutegravir (mg)	100	100	100	100	100	100	100	100	100
2.	Polyvinyl alcohol (mg)	200	200	200	200	200	200	200	200	200
3.	Ethyl cellulose (mg)	100	100	100	150	150	150	200	200	200
4.	Speed of rotation (RPM)	14000	16000	18000	14000	16000	18000	14000	16000	18000

5.	Dichloromethane (ml)	20	20	20	20	20	20	20	20	20
6.	Distilled water (ml)	100	100	100	100	100	100	100	100	100

Table 8: Formulation strategy

4. Evaluation of formulated batches:

a. Physical evaluation:

Physical evaluation factors, including color, odor, and appearance, were assessed for each prepared batch. All batches were having a uniform appearance and there was no odour difference in between any batch as all batches were characteristic in odour. Colour difference was not observed among. Table 9 listed the outcomes for each batch..

Batches	Colour	Odour	Appearance
F1	Off-white	Characteristic	Fine particles
F2	Off-white	Characteristic	Fine particles
F3	Off-white	Characteristic	Fine particles
F4	Off-white	Characteristic	Fine particles
F5	Off-white	Characteristic	Fine particles
F6	Off-white	Characteristic	Fine particles
F7	Off-white	Characteristic	Fine particles
F8	Off-white	Characteristic	Fine particles
F9	Off-white	Characteristic	Fine particles

Table 9: Physical evaluation of Nanoparticles

b. Determination of practical yield:

The formula was used to compute the practical yield of the prepared Dolutegravir nanoparticles, and the results were given in Table 10.

$$\text{Practical yield} = \frac{\text{Practical weight of nanoparticles}}{\text{Theoretical weight of (Drug + Polymer)}} \text{ X } 100$$

Batches	Practical mass (Nanoparticles) mg	Theoretical yield (Drug + Polymer) mg	% Practical yield
F1	150.32	200	75.16
F2	156.44	200	78.22
F3	171.26	200	85.63
F4	193.15	250	77.26
F5	206.1	250	82.44
F6	210.9	250	84.36
F7	210.36	300	70.12
F8	234.57	300	78.19
F9	237.96	300	79.32

Table 10: Determination of percent practical yield

c. Determination of Entrapment efficiency:

The F9 formulation had the maximum entrapment efficiency (94.16), while the F1 formulation had the lowest drug entrapment. This could be because variations in the concentration of the polymer caused variations in entrapment efficiency. The produced nanoparticles were shown to have a high drug entrapment effectiveness, ranging from 80.35% to 94.16%.

Batches	Entrapment efficiency (%)
F1	80.35
F2	84.88
F3	87.63
F4	85.58
F5	88.65

Table 11: Determination of percent Entrapment efficiency

d. In vitro dissolution study:

In vitro drug release study of the prepared Dolutegravir nanoparticles was carried out

using dialysis bag diffusion method. Amount of drug released in different time intervals were observed. The percent cumulative drug release for all the batches were found in the range of 83.57 – 98.75 %. As the concentration of polymer in nanoparticles increases percent cumulative drug release also increases with decrease in time. According to in vitro dissolution data, the F6 batch had the highest cumulative drug release percentage (98.75%) compared to the other batches. Table 12 and Figure 8 presented the findings.

Time (Hrs.)	Batches	% Cumulative Drug Release (%)								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1		15.46	14.588	12.445	13.688	11.288	13.455	11.388	12.455	10.355
2		29.277	27.114	21.669	28.116	26.166	27.669	23.166	23.366	21.669
3		45.999	44.668	38.24	41.05	35.005	37.04	34.35	35.67	32.288
4		58.777	59.778	52.27	54.06	56.336	49.27	42.46	40.772	47.741
5		75.155	74.588	70.39	65.778	67.788	61.559	55.28	49.16	55.25
6		90.266	89.466	78.13	77.28	75.28	75.13	68.78	59.25	68.03
7		94.666	93.92	85.34	85.06	86.06	85.64	75.25	71.67	74.92
8		92.216	94.155	95.96	98.75	89.36	85.61	83.57		

Table 12: Determination of % drug diffusion

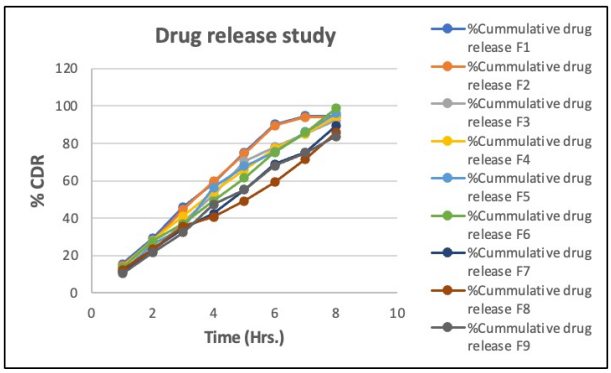


Figure 8: % Drug release study

5. Optimization of prepared nanoparticles:

Design Expert 7.0 software was used to investigate how independent variables affected answers. An experimental design pattern was created for nine potential batches of nanoparticles loaded with dolutegravir. The software recommended and tested a number of models, including Linear, 2FI, Quadratic, and Cubic, that fit well for analysis of variance (ANOVA). For each of the dependent variables, regression polynomials were computed. For every single dependent variable or response, mathematical models were created and written as equations.

Runs	Factor1	Factor 2	Response 1	Response 2
	A: Speed of rotation (RPM)	B: Ethyl cellulose (mg)	Particle size (nm)	Entrapment efficiency (%)
1	1600	100	210.9	84.88
2	1800	200	201.7	94.16
3	1600	200	268.9	92.88
4	1400	150	304.5	85.58
5	1400	200	315.2	90.35

6	1800	100	195.4	87.63
7	1800	150	135.3	90.05
8	1600	150	245.7	88.65
9	1400	100	254.3	80.35

Table 13: The layout of Actual Design

Results for Particle size:

1. Fit Summary: The "Linear vs. Mean" option was recommended by the Design-Expert software following the application of fit summary to the data.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	504999.7	1	504999.7			
Linear vs Mean	22060.93	2	11030.47	16.53015	0.0036	Suggested
2FI vs Linear	745.29	1	745.29	1.143619	0.3338	
Quadratic vs 2FI	426.3511	2	213.1756	0.225812	0.8103	
Cubic vs Quadratic	2494.787	2	1247.393	3.697794	0.3451	Aliased
Residual	337.3344	1	337.3344			
Total	531064.4	9	59007.16			

Table 14: Fit summary table for Particle size

1. **ANOVA for Particle size:**

To determine which factors were significant and which were not, the analysis of variance (ANOVA) was used. The following are the particle size ANOVA results.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	22060.93	2	11030.47	16.53015	0.0036	significant
A-Speed of rotation	19448.43	1	19448.43	29.14523	0.0017	
B-Ethyl cellulose	2612.507	1	2612.507	3.915078	0.0952	
Residual	4003.762	6	667.2937			
Cor Total	26064.7	8				

Table 15: ANOVA table for Particle size

The model's significance is implied by its F-value of 16.53. A "Model F-Value" this high has a mere 0.36% probability of being caused by noise. Model terms are considered significant when "Prob > F" values are less than 0.0500. A and B are important model terms in this instance.

3 Fit Statistics for Particle size

Std. Dev.	25.83	R-Squared	0.8464
Mean	236.88	Adj R-Squared	0.7952
C.V. %	10.91	Pred R-Squared	0.6228
PRESS	9831.62	Adeq Precision	10.433

Table 16: Fit statistics for Particle size

The "Adj R-Squared" of 0.7952 and the "Pred R-Squared" of 0.6228 agree fairly well. The signal to noise ratio is measured using "Adeq Precision." It is preferable to have a ratio larger than 4.

An appropriate signal is indicated by a ratio of 10.433. The design space can be navigated using this concept.

4. Final Equation in Terms of coded Factors for Particle size:

Particle size	=
+236.88	
-56.93	* A
+20.87	* B

Table 17: Final equation in terms of coded factors

Predictions regarding the response for specific amounts of each element can be made using the equation in terms of coded factors.

5. Graphical Presentation:

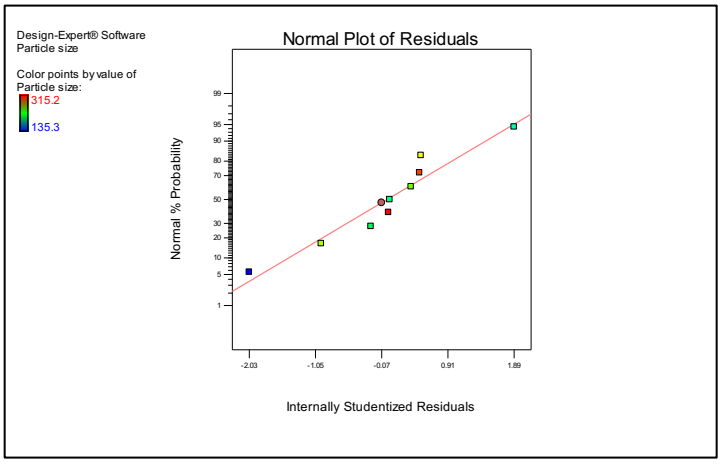


Figure 9: Normal % Probability plot of Particle size

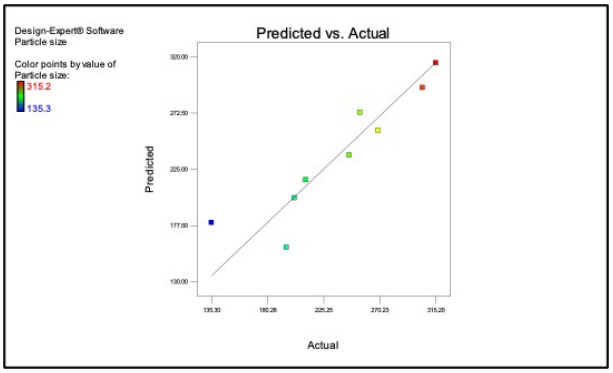


Figure 10: Predicted Vs Actual plot for Particle size

5. Model Graphs for Particle size:

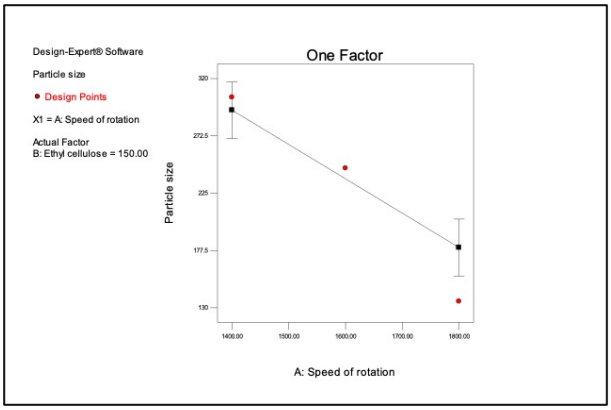


Figure 11: Effect of Speed of rotation on particle size

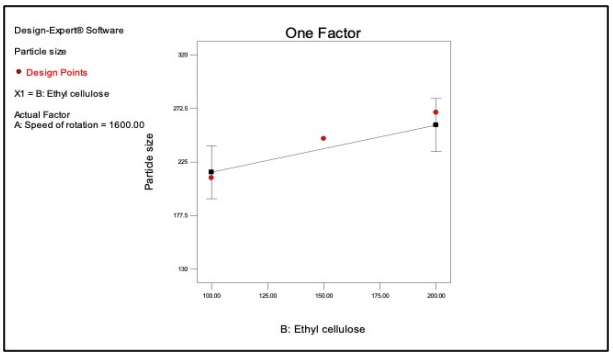


Figure 12: Effect of Ethyl cellulose concentration on Particle size

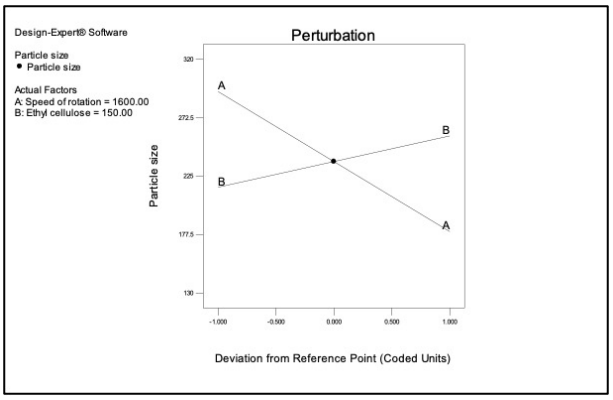


Figure 13: Effect of Speed of rotation and ethyl cellulose on Particle size

In conclusion, the concentration of ethyl cellulose and rotation speed affect the size of the nanoparticles. The size of the nanoparticles decreases with increasing rotation speed and grows with increasing ethyl cellulose concentration.

Results for Entrapment efficiency:

1. Fit Summary: The "2FI vs. Linear" option was recommended by the Design-Expert software after the data was entered and fit summary was applied.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	70141.99	1	70141.99			
Linear vs Mean	140.6391	2	70.31954	87.76402	< 0.0001	
2FI vs Linear	3.010225	1	3.010225	8.374854	0.0340	Suggested
Quadratic vs 2FI	1.385894	2	0.692947	5.05449	0.1095	
Cubic vs Quadratic	0.408617	2	0.204308	76.5359	0.0806	Aliased
Residual	0.002669	1	0.002669			
Total	70287.44	9	7809.715			

Std. Dev.	0.60	R-Squared	0.9876
Mean	88.28	Adj R-Squared	0.9802
C.V. %	0.68	Pred R-Squared	0.9626
PRESS	5.44	Adeq Precision	33.435

Table 18: Fit summary table for Entrapment efficiency

1. **ANOVA for Entrapment efficiency:**

To determine which factors were significant and which were not, the analysis of variance (ANOVA) was used. The results of ANOVA for the entrapment efficiency are as following.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	143.6493	3	47.8831	133.2173	< 0.0001	significant
A-Speed of rotation	40.35227	1	40.35227	112.2655	0.0001	
B-Ethyl cellulose	100.2868	1	100.2868	279.0115	< 0.0001	
AB	3.010225	1	3.010225	8.374854	0.0340	
Residual	1.797181	5	0.359436			
Cor Total	145.4465	8				

Table 19: ANOVA table for Entrapment efficiency

The Model F-value of 133.22 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B and AB are significant model terms.

1. **Fit Statistics for Entrapment efficiency:**

Table 20: Fit statistics for Entrapment efficiency

The "Pred R-Squared" of 0.9626 is in reasonable agreement with the "Adj R-Squared" of 0.9802. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. ratio of 33.435 indicates an adequate signal. This model can be used to navigate the design space.

2. **Final Equation in Terms of coded Factors for Entrapment efficiency:**

Entrapment efficiency	=
+88.28	
+2.59	* A
+4.10	* B
-0.85	*A*B

Table 21: Final equation in terms of coded factors

For certain levels of each element, the reaction can be predicted using the equation in terms of coded factors.

Graphical Presentation:

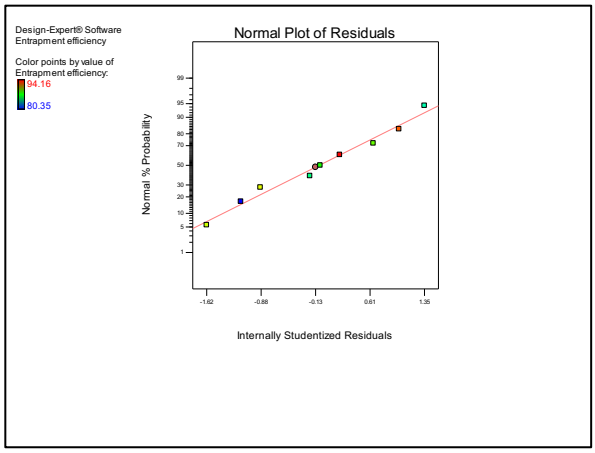


Figure 14: Normal % Probability plot of Entrapment efficiency

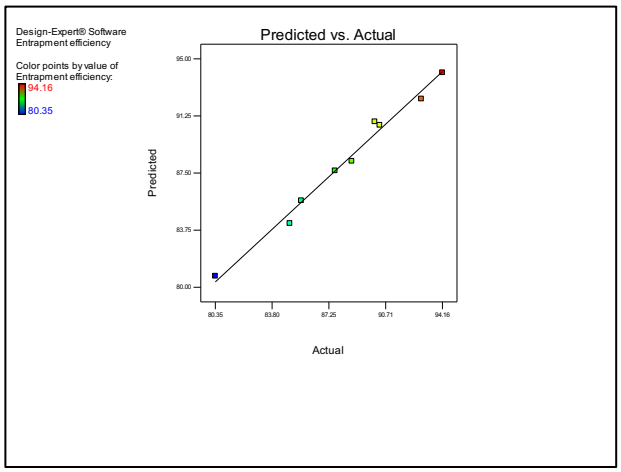


Figure 15: Predicted Vs Actual plot for Entrapment efficiency

3. Model Graphs for Entrapment efficiency:

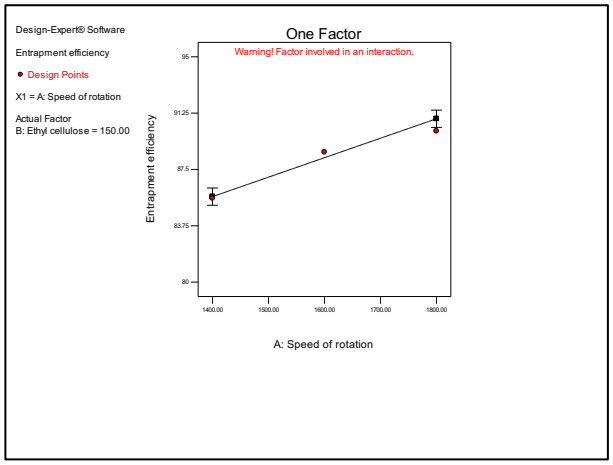


Figure 16: Effect of Speed of rotation on Entrapment efficiency

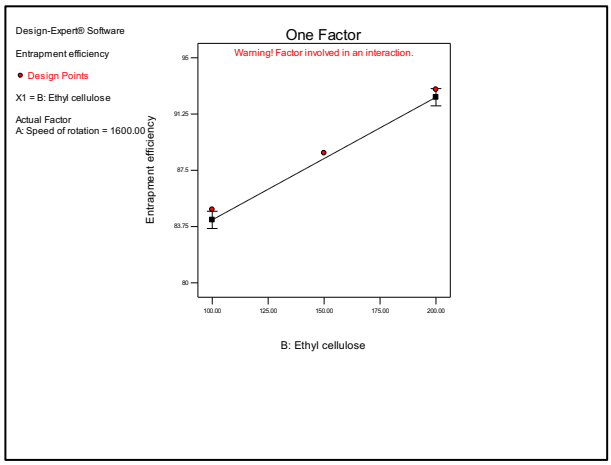


Figure 17: Effect of Ethyl cellulose concentration on Entrapment efficiency

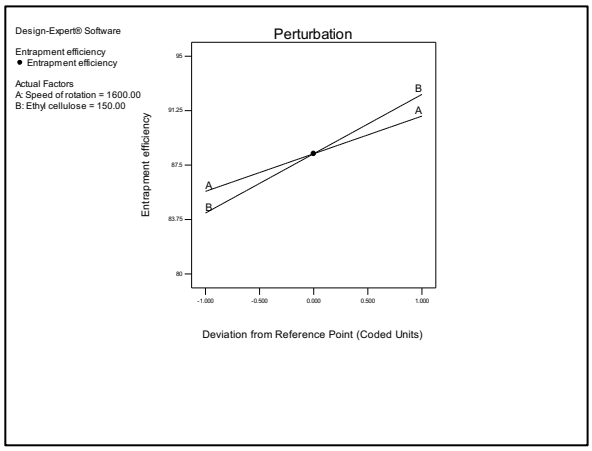


Figure 18: Effect of Speed of rotation and ethyl cellulose on Entrapment efficiency

In conclusion, the concentration of ethyl cellulose and rotational speed have an effect on the effectiveness of nanoparticle entrapment. The entrapment effectiveness of nanoparticles rises with increasing rotation speed and with increasing ethyl cellulose concentration.

Sr. No.	Independent variables	Particle size	Entrapment efficiency
1	Speed of rotation (RPM)	Inversely proportional (As speed of rotation increases particle size decreases)	Directly proportional (As speed of rotation increases entrapment efficiency increases)
2	Concentration of Ethyl cellulose (mg)	Directly proportional (As Conc. of ethyl cellulose increases particle size increases)	Directly proportional (As Conc. of ethyl cellulose increases entrapment efficiency increases)

Table 22: Summary for effect of independent variable on responses

Conclusion: The F6 batch, which had low particle size, high entrapment efficiency, and sustained drug release for eight hours, was chosen as the optimum batch based on data from batch evaluation and the factorial design model analysis.

Result for particle size:

The particle size is one of the most important parameter for the characterisation of nanoparticles. The average particle sizes of the prepared Dolutegravir nanoparticles were measured using Malvern zeta sizer. Result was mentioned under figure 19.

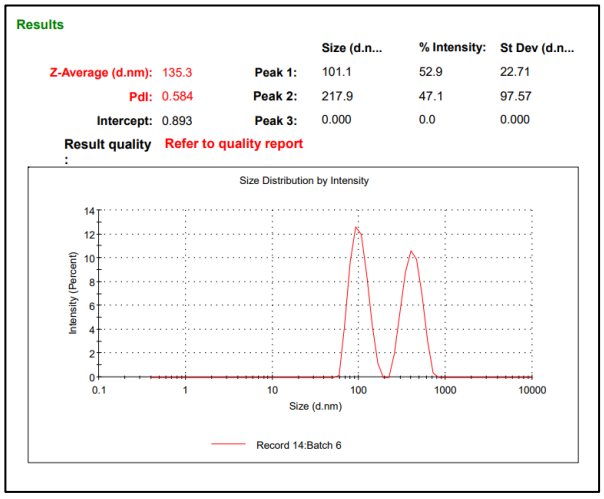
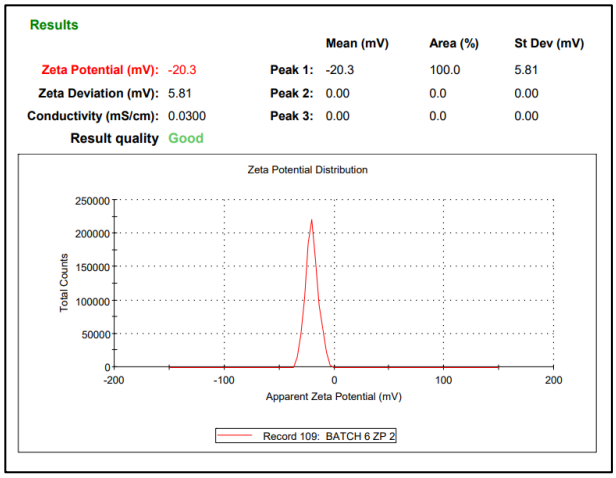


Figure 19: Particle size analysis for optimized batch (F6)

Result for Zeta potential:

The Malvern zeta-sizer device was used to calculate zeta potential. Zeta potential analysis is used to determine the particles' surface charge in order to determine their stability during storage. The colloidal stability is predicted by the size of the zeta potential. High levels of stability are usually found in nanoparticles with zeta potential values more than +20 mV or less than -20 mV. Dolutegravir nanoparticles were discovered to have a peak area of 100% intensity and a zeta potential of -20.3mV.



References

1. World Health Organization. HIV/AIDS fact sheet**.** Geneva: World Health Organization; 2023.
2. Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. Lancet. 2013;382(9903):1525–33.
3. Flexner C. Antiretroviral agents and treatment of HIV infection. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J, editors. Harrison's principles of internal medicine. 20th ed. New York: McGraw-Hill Education; 2018. p. 1235–48.
4. Raffi F, Rachlis A, Stellbrink HJ, Hardy WD, Torti C, Orkin C, et al. Once-daily dolutegravir versus raltegravir in antiretroviral-naïve adults with HIV-1 infection: 48-week results from the SPRING-2 study. Lancet. 2013;381(9868):735–43.
5. Walmsley SL, Antela A, Clumeck N, Duiculescu D, Eberhard A, Gutiérrez F, et al. Dolutegravir plus abacavir–lamivudine for the treatment of HIV-1 infection. N Engl J Med. 2013;369(19):1807–18.
6. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database. Nucleic Acids Res. 2018;46(D1):D1074–82.
7. Anderson PL, Kakuda TN, Lichtenstein KA. The cellular pharmacology of nucleoside- and nucleotide-analogue reverse-transcriptase inhibitors and its relationship to clinical toxicities. Clin Infect Dis. 2004;38(5):743–53.

8. Rawat M, Singh D, Saraf S, Saraf S. Nanocarriers: promising vehicles for bioactive drugs. Biol Pharm Bull. 2006;29(9):1790–8.
9. Mohanraj VJ, Chen Y. Nanoparticles: a review. Trop J Pharm Res. 2006;5(1):561–73.
10. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles-based drug delivery systems. Colloids Surf B Biointerfaces. 2010;75(1):1–18.
11. Rowe RC, Sheskey PJ, Quinn ME, editors. Handbook of pharmaceutical excipients. 6th ed. London: Pharmaceutical Press; 2009.
12. Bodmeier R, McGinity JW. Polylactic acid microspheres containing quinidine base and quinidine sulphate prepared by the solvent evaporation technique. J Microencapsul. 1987;4(4):279–88.
13. Gibson M. Pharmaceutical preformulation and formulation. 2nd ed. Boca Raton: CRC Press; 2015.
14. Crowley MM, Zhang F, Repka MA, Thumma S, Upadhye SB, Battu SK, et al. Pharmaceutical applications of hot-melt extrusion: Part I. Drug Dev Ind Pharm. 2007;33(9):909–26.
15. Song KC, Lee HS, Choung IY, Lee CH, Kim KS, Ahn Y. The effect of organic phase solvents on the particle size of poly(D,L-lactide-co-glycolide) nanoparticles. Colloids Surf A Physicochem Eng Asp. 2006;276(1–3):162–7.
16. Montgomery DC. Design and analysis of experiments. 8th ed. Hoboken (NJ): John Wiley & Sons; 2013