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FABRICATION AND STATISTICAL OPTIMIZATION OF LIPID-BASED NANOPARTICLES FOR THE ENCAPSULATION OF DOLUTEGRAVIR SODIUM

Sreethu K. Sreedharan and C. U. Amrutha *

Department of Pharmaceutics, Grace College of Pharmacy, Palakkad - 678004, Kerala, India.

Keywords:

Lipid based Nanoparticles, Hot melt emulsification sonication method, Dolutegravir sodium, HIV treatment, optimization

Correspondence to Author:
C. U. Amrutha

Associate Professor,
Department of Pharmaceutics,
Grace College of Pharmacy,
Palakkad - 678004, Kerala, India.

E-mail: amruthaunni03@gmail.com

ABSTRACT: Dolutegravir, an integrase strand transfer inhibitor (INSTI), is a BCS class II, poorly soluble antiretroviral medication that has been licenced by the FDA for the treatment of HIV infections. DTG has a number of drawbacks, including limited oral bioavailability (16%), hepatotoxicity, and instability in an acidic environment. Due to the medication's limited solubility, 84% of the dose is eliminated from the body after oral administration in an unaltered state. Lipid-based nanoparticles have been chosen for the current research study as a viable drug delivery technology to help with the aforementioned challenges. Melt emulsification sonication procedure was used with CAPMUL MCM EP, Glyceryl monostearate, TWEEN 80, SPAN 80, and other ingredients, a total of six formulations were created. The formulations' particle size, polydispersity index, zeta potential, drug concentration, drug entrapment effectiveness, *in-vitro* release, and other factors were assessed. The prepared formulations demonstrated zeta potential of -11.78 to-26.6, in-vitro drug release of between 62 and 80.32 percent, and particle sizes varying from 155 to 205 nm. The best formulation, according to the early trial, was F2. Particle size Y1 and *in-vitro* drug release Y2 were two dependent variables, and F2 was fitted to a 2^2 factorial design with two independent factors, concentration of span 80 - X1 and sonication duration - X2. 4 runs were compared, and code R2 was determined to be the most optimal formulation. The improved dolutegravir-loaded Nano formulation offers an adequate release and may be appropriate for large-scale manufacturing with improved therapeutic outcome and patient compliance.

INTRODUCTION: Lipid-based nanoparticles are a promising delivery system for many treatments [1]. It is a submicron colloidal dispersion of drug particles that is mostly utilized to get beyond the constraints of colloidal carriers like emulsions and liposomes since it has a great releasing profile and outstanding physical stability [1, 2]. One of the cutting-edge technologies, lipid-based drug delivery systems (LBDDS), aims to solve the problems of poor solubility and bioavailability.

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The drug's bioavailability may be boosted by encapsulating or solubilizing it in lipid excipients, which can promote solubilization and absorption. The development of lipid-based formulations as a method for drug delivery has been aided by the availability of novel lipid excipients with acceptable regulatory and safety profiles along with their capacity to increase oral bioavailability [10].

A variety of lipid-based drug delivery systems have been successful due to the proper choice of lipid vehicles, formulation techniques, and thoughtful system design. To improve solubility, all medications in Biopharmaceutical Classification System (BCS) classes II and IV are susceptible to nano-formulation innovation [15]. Dolutegravir sodium (DTG) is a BCS Class II poorly soluble

antiretroviral medication used to treat HIV infections. The acquired immunodeficiency syndrome (AIDS) is believed to be caused by the human immunodeficiency virus (HIV), which is known to promote the ongoing degeneration of the host immune system (AIDS) [5]. The capacity to explore and defend ranges for formulation and processing components is greatly enhanced by an optimization technique. The definition of the word "optimize" is "to perfect," which refers to selecting the best element from a group of viable options. 2^2 factorial designs were conducted in order to get the optimum formulation, which might produce the best solution to the study's problem and require fewer experiments. Two independent and four dependent variables were assessed during the experimental trials, which were conducted at 4 different combinations. As a result of their poor aqueous solubility and limited bioavailability following oral administration, the majority of new drugs have poor absorption [20].

The diminution of drug particle size and salt formation through the use of surfactants, cyclodextrins, liposomes, or nanoparticles has been defined as a number of methods to speed up drug dissolution [1]. Lipid-based nanoformulations are a comparatively new strategy for drugs with poor solubility (LNPs) [16]. In order to improve patient compliance and minimise the drug's dose while targeting it at the desired location. Antiretroviral therapy is unquestionably significant, but it has been constrained by a number of issues, including its intrinsic toxicity, a lack of efficacy, and drug resistance [13]. Some of these problems have been reduced by the creation and recent approval of new or enhanced medications, but the remarkable capacity of HIV to withstand the new therapeutic choices has limited achievement [13].

Additionally, the physical-chemical deficiencies of the majority of these antiretroviral medications (such as poor solubility, permeability, and stability) hinder ideal biodistribution and sustained antiretroviral effect, which negatively impacts clinical outcome [6]. Countless novel and enhanced dosage forms and delivery systems have been recommended in the literature as fixes to these issues. In particular, a number of nanotechnology-based delivery methods have been created to enhance HIV therapy, including polymeric nanoparticles, solid lipid nanoparticles (SLNs), liposomes, nano emulsions, dendrimers, and drug conjugates (e.g. with low-density lipoproteins or peptides) [17]. The main objectives of this research effort are to fabricate and statistically optimise lipid-based nanoparticles containing dolutegravir sodium in order to increase their bioavailability and enhance their therapeutic profile.

MATERIALS AND METHODS: Dolutegravir sodium (mylanpvt, bangalore), Capmul MCM EP, glyceryl mono stearate (continental, new delhi), Tween 80, Span 80, Sodium Cholate, Propyleneglycol and PEG 6000 (Yarrow Chem products, Mumbai).According to I.P., B.P., U.S.P., or internal specifications, all of the excipients used in pharmaceutical products were authorized, and all other chemicals were of analytical grade.

METHODS:

Stage 1: Preparation of Dolutegravir Sodium Loaded Nanoparticles for Preliminary Trial: The hot melt emulsification sonication method was used to create various nanoparticle compositions containing dolutegravir sodium. The formulation of LNPs included the use of solid and liquid lipids in various concentrations (80: 20, 70: 30), along with a surfactant mixture [(Tween 80: Span80), (Poloxamer 407: Span 80), (70: 30)]. A total of six batches were generated for the preliminary trial. Using a vortex cyclomixer, the drug was dissolved and distributed as a liquid lipid. After adding propylene glycol, BHT, and solid lipids, the melted solid lipids were mixed with an oil-soluble surfactant called SPAN 80. By mixing in tween 80 and PEG 6000, a water-soluble surfactant, in the same quantity as needed and heating the mixture to the same temperature as the oil phase, the aqueous phase was simultaneously created.

The drug-containing melted lipid phase was rapidly transferred into the aqueous phase, and the mixture was stirred for 20 minutes at 700 rpm on a magnetic stirrer. To create nanoemulsion from the produced coarse emulsion, a probe sonicator was used. After the lipid solidified at room temperature, the nano emulsion was then transformed into LNPs.

Stage 2: Statistical Optimization of Best Preliminary Trial batch: Based on the evaluation parameters the F-2 batch was found to be best

preliminary trial formulation. The batch was statistically optimized using a 2^2 factorial design approach. The concentration of Solid-liquid lipid ratio (X1) and the sonication time (X2) were taken as independent variables. Two responses (particle size (Y1), and *In-vitro* drug release (Y2) was used as the dependent variables [15].

Stage 3 Formulation of Nanoparticles Loaded Dry Powder: Lipid based Nanoparticles weight equivalent to 2.5 mg of dolutegravir containing formulations were subjected to freeze drying process and can be used for paediatric dosage form in the future [20].

Characterization of Nanoparticles for Preliminary Trial Batches:

Compatibility Study by FTIR: Drug excipients compatibility study was assessed by FT-IR method. The spectrum was assessed for the pure drug, drug and excipients of nanoparticles and nanoparticle loaded dry powder.

Particle size and Poly-dispersity Index: The mean particle size and size distribution or polydispersity index of the prepared nanoparticles were analysed using a zeta sizer. The samples are placed in the analyser chamber and the readings are carried out at a 90° angle with respect to the incident beam in 0.75ml capacity disposable cuvettes [10].

Entrapment Efficiency (%): The percentage encapsulation of all the prepared formulation was estimated by measuring the amount of unbound drug in the formulation by ultra-centrifugation method. The entrapment efficiency of the prepared nanoparticles was calculated by the following formula:

Entrapment Efficiency (%) = Total Drug-Drug in supernatant liquid / Total drug × 100

Zeta Potential: The zeta potential of Nano suspension is measured using Zeta sizer Nano ZS at 25±0.5°C. A potential of ±150 mV is set in the instrument in 0.75ml capacity disposable cuvettes [11].

***In-vitro* Drug Release Study:** By using the dialysis bag diffusion method, different nano formulations were *in-vitro* released. Dialysis tubes will serve as the dialysis bag. Dialysis tubes are typically 4-5 centimetres long. The pH 6.8 phosphate buffer was added, the sac was hermetically sealed, and the contents were then drained to check for leaks.

Following that, it was suspended in a stoppered vessel holding 100 ml of pH 6.8 phosphate buffer, which acted as the receptor compartment. The media should be at a pace of 100 rpm and a temperature of 370°C + 0.50°C. 1 ml of the sample was taken from the receptor compartment at periodic times and used to figure out how much drug was released.

At each time point, a new buffer was added to the receptor section. The samples were taken at 1, 2, 3, 5, 6, 7, and 8 hours. For every formulation that was developed as diffusion tests and sample analysis were done.

Collected samples were appropriately diminished in pH 6.8 phosphate buffer before being analysed at 258 nm with pH 6.8 phosphate buffer functioning as the reference standard on a UV spectrophotometer.

TABLE 1: LAYOUT OF INDEPENDENT VARIABLES FOR OPTIMIZATION

Factors (Independent)	Levels		
	+1	**0**	**-1**
X1 (Concentration. of surfactant)	6%	5%	4%
X2 (Sonication time)	30	20	10

TABLE 2: LAYOUT AND RESULTS OF DOLUTEGRAVIR SODIUM NP – DEPENDENT VARIABLES

Runs	X_1	X_2	Y1	Y_2
R1	-1	-1	170 ± 4	74.4 ± 1
R2	-1	+1	165 ± 3	80.25 ± 1
R3	+1	-1	175 ±2	78.15 ± 2
R4	+1	+1	171 ± 4	76.21± 4

Note: Y1= particle size (nm) Y2= *in-vitro* drug release (%).

Characterization of Optimized Formulation: The statistically optimized formulation (R2) was characterized for the following parameters.

Drug Release Kinetic Study: To comprehend the rate of drug release mechanism of the prepared formulations, the obtained dissolution data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas equations [16].

Scanning Electron Microscopic Study (SEM): SEM was used to examine the morphology of the optimised nanoparticles (R2). (S-4800, Hitachi Technologies Corporation, Japan). 1.5 kV of acceleration voltage is used to run the scanning electron microscope [18, 19].

RESULTS AND DISCUSSION: Tables 1-3 and **Fig. 1-7** contain a summary of all the findings. The drug was compatible with all of the excipients, according to the FTIR analysis. By implementing the hot melt emulsification sonication technique, the nanoparticles were created. At pH 6.8, the formulation's maximum was discovered to be 258. The melting point of formulation was found to be 300°C. There was a slight increase in the mass of the API which indicated that the drug is slightly hygroscopic in nature.

The slope at pH 6.8 buffer was found to be 0.0592 and R2 was 0.999. In the drug's and excipients' FTIR spectra, all significant peaks can be found. The study's findings show that there was no difference in the main peaks of dolutegravir sodium in the FT-IR spectrum of the drug and excipients. The fact that all of the drug's main peaks were visible on the blend, i.e., there is no potential for a drug-excipient interaction **Fig. 1, 2.**

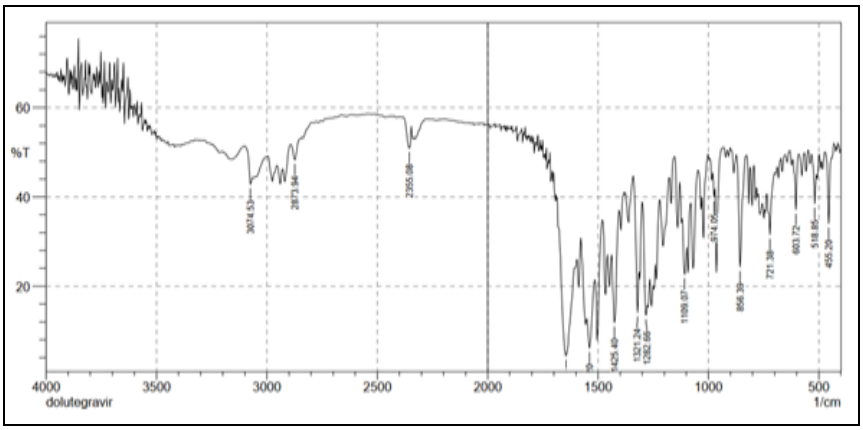

FIG. 1A: FTIR SPECTROSCOPY FOR DOLUTEGRAVIR SODIUM PURE

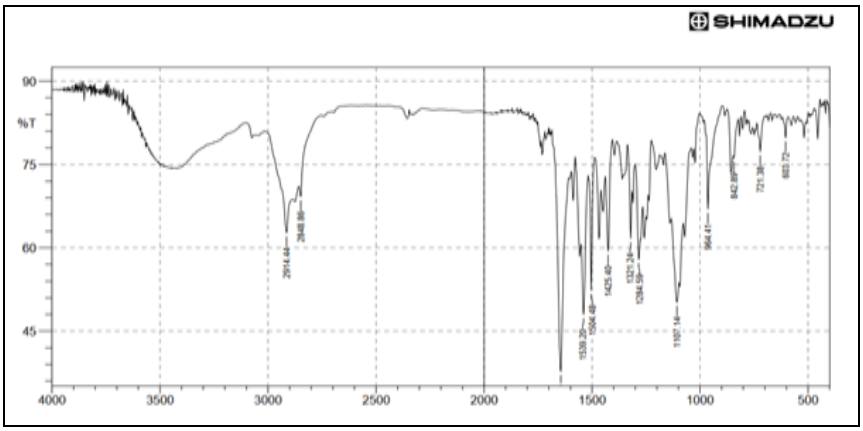

FIG. 1B: FTIR SPECTROSCOPY OF DOLUTEGRAVIR LOADED NANOPARTICLES + EXCIPIENTS

The prepared nanoparticles were subjected to primary evaluation studies. The formulations entrapment efficiencies fell between the ranges of 72.5 and 88.09. Greater entrapment efficiency was observed in the F2, F4, F5, and F6 formulas because it was discovered that the entrapment efficiency increased with increasing lipid and surfactant concentrations. The particulate size ranged from 155 to 205 nm.

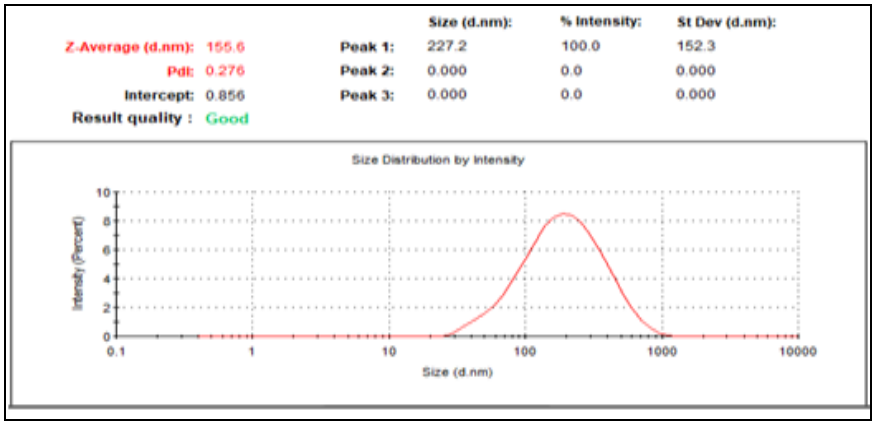

FIG. 2: PARTICLE SIZE AND PDI OF F2

From the particle size studies, the zeta potential values were in the range of -26.6 - 11.78 mV

TABLE 3: RESULTS OF EVALUATION OF DOLUTEGRAVIR LOADED NANOPARTICLES – PRELIMINARY TRIAL

S. no.	Parameters	Formulation Code					
		F1	F2	F3	F4	F5	F6
1.	Drug content	95 ± 0.21	99 ± 0.25	96.3 ± 0.03	96.8 ± 0.17	94.9 ± 1.2	98.3 ± 0.2
2.	Entrapment efficiency (%)	75.42 ± 3	88.09 ± 5	72.5 ± 4	85.6 ± 7	78.2 ± 6	74.2 ± 4
3.	Particle size (nm)	195 ± 3	175 ± 2	180.7 ± 5	186.9 ± 4	205 ± 6	185.2 ± 7
4.	Polydispersity Index	0.198±2	0.114±2	0.139±5	0.193±3	0.167±4	0.280±2
5.	Zeta Potential (mV)	-19.8	-26.6	-14.11	-15.19	-14.28	-11.78

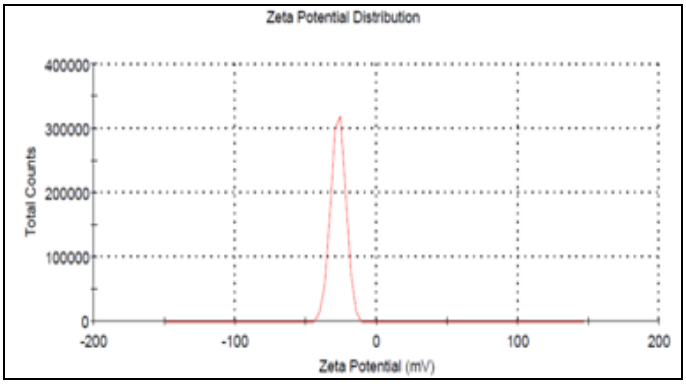

FIG. 3: ZETA POTENTIAL OF F2 FORMULATION

As a result, it can be concluded that lipid-based nanocarriers revealed a small, homogeneous particle size of 155 nm and a polydispersity index; the batch with the longest sonication time demonstrated a better polydispersity index than the other batches. 94.9 to 99.9% of the substance contained drugs.

In F2, F4, and F6 formulations, the drug content was raised. As a consequence of the higher surfactant concentration, the nanoparticles' drug content increased, according to the findings. Indicating a consistent size distribution, the polydispersity index was within 0.276. In the formulation with the best entrapment, the zeta potential was in the range of -26.6.

***In-vitro* Release Study:** The *in-vitro* drug release is affected by both the composition of the nanoparticles as well as the composition of the dry powder.

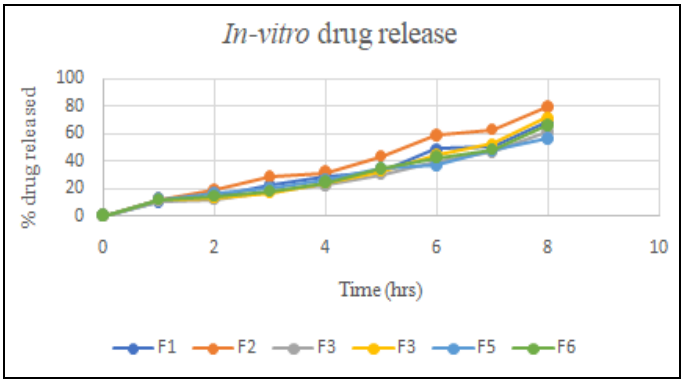

FIG. 4: *IN-VITRO* DRUG RELEASE PROFILE FOR ALL PREPARED FORMULATION OF DOLUTEGRAVIR LOADED NANOPARTICLES

The evaluation tests revealed that F2 was the most effective formulation. Particle size (Y_1) and in vitro drug release (Y_2) were response factors when F2 was fitted to a 2^2 factorial design with two independent variables, concentration of span 80 and sonication time (X_1 and X_2). The best formulation, R2, had a 155 nm particle size and an *in-vitro* drug release of 80.4%, according to the results.

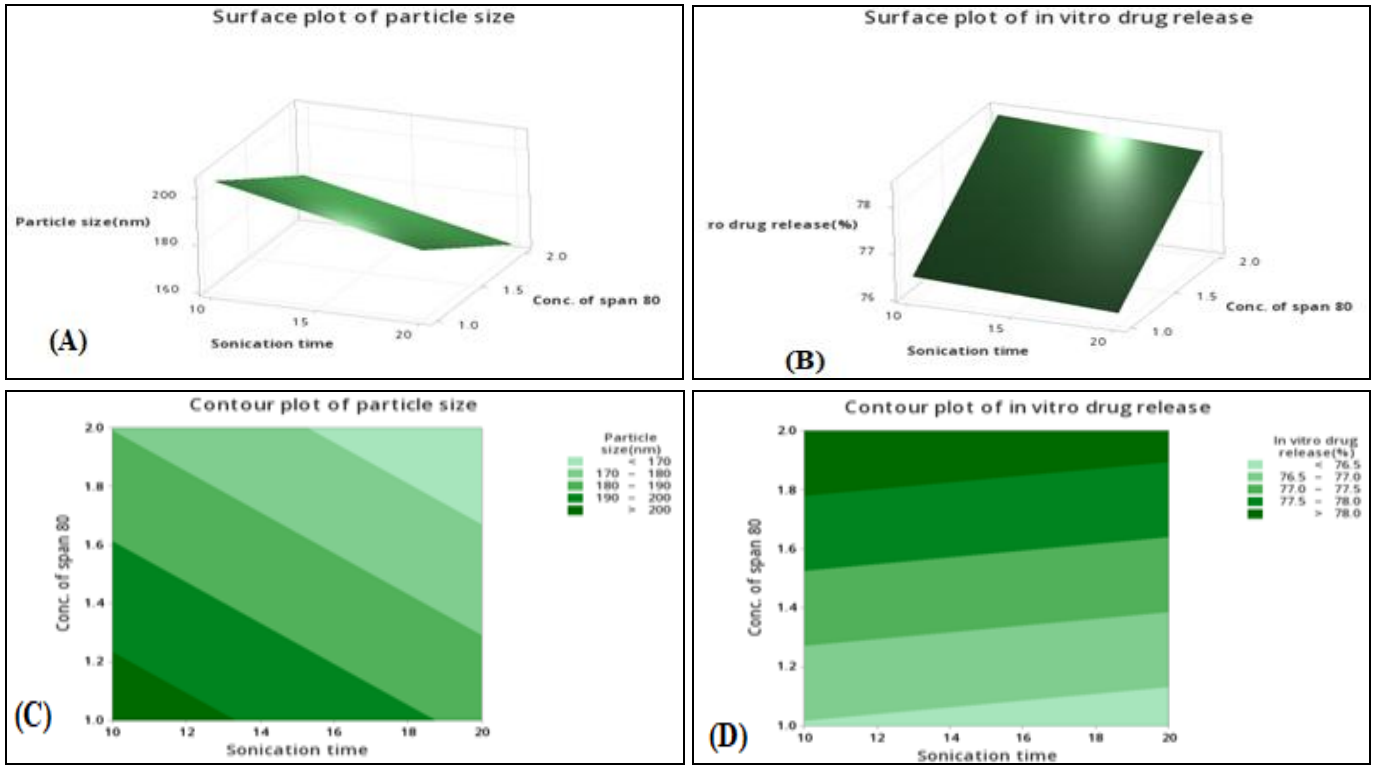

FIG. 5: (A) SURFACE PLOT OF Y1 (PARTICLE SIZE), (B) SURFACE PLOT OF Y2 *(IN-VITRO* RELEASE), (C) CONTOUR PLOT OF PARTICLE SIZE, (D) CONTOUR PLOT *OF IN-VITRO* DRUG RELEASE

The optimized formulation was exposed to kinetic study, SEM study and stability study. The kinetic study indicated that the formulation is controlled release with Super Case II transport as the n value obtained from the Korsemeyerpeppas was 1.4579.

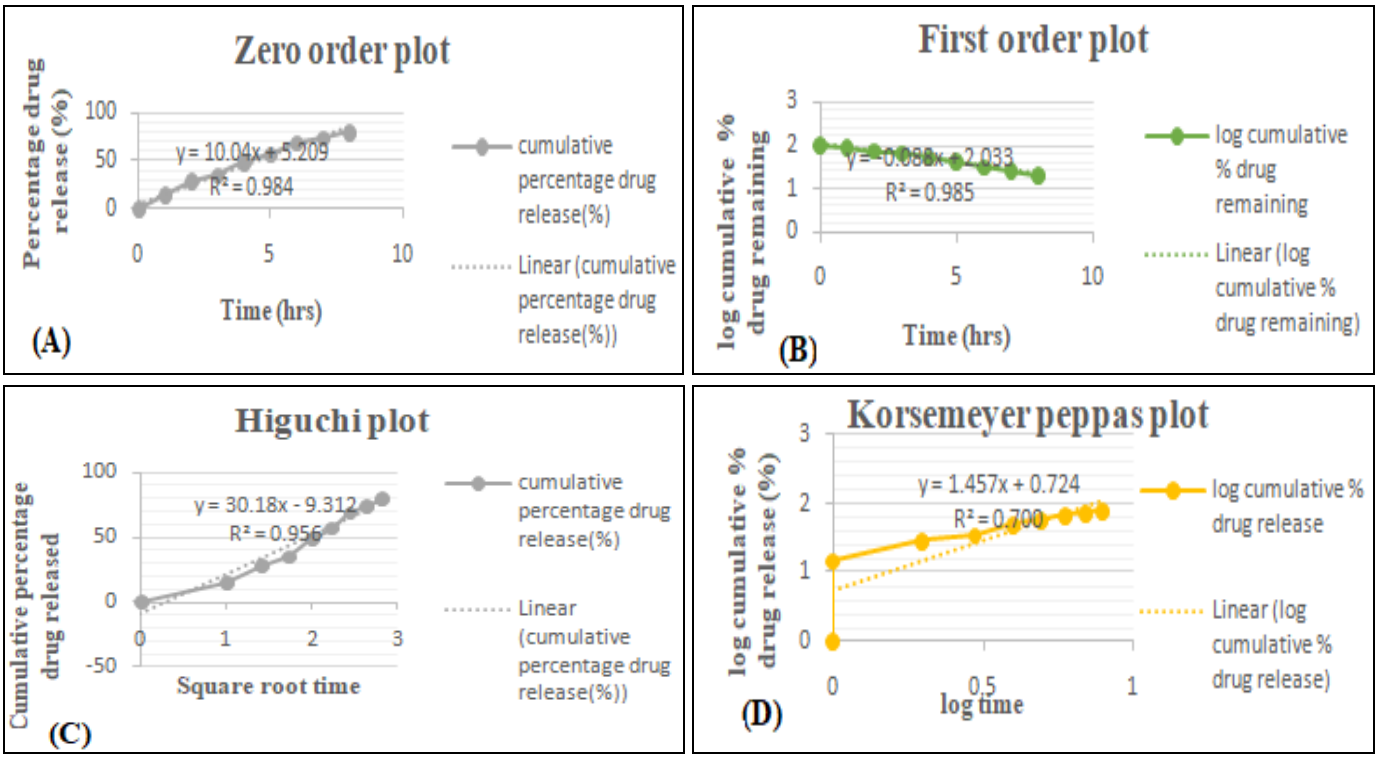

FIG. 6: (A) ZERO ORDER PLOT FOR OPTIMIZED FORMULATION, (B) FIRST ORDER PLOT FOR OPTIMIZED FORMULATION, (C) HIGUCHI PLOT FOR THE OPTIMIZED FORMULATION, (D) KORSEMEYERPEPPAS PLOT FOR THE OPTIMIZED FORMULATION

Scanning electron microscopic image indicated that particles displayed spherical shape and smooth surface. There are some differences in surface morphology of nano particles, but generally spherical morphology with the presence of spherical disks was observed.

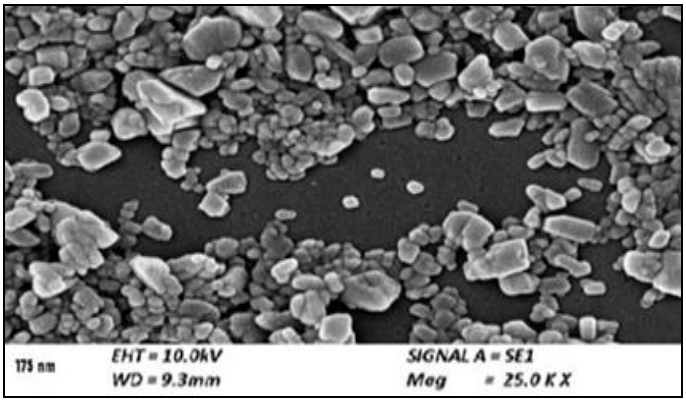

FIG. 7: SCANNING ELECTRON MICROSCOPIC IMAGE OF SURFACE OF OPTIMIZED FORMULATION (R2)

Stability study showed that there are no significant changes in the dissolution pattern. The results of the 2^2 factorial designs revealed that R2 formulation is suitable for large scale manufacturing of lipid based nano formulation containing dolutegravir nanoparticles.

CONCLUSION: Dolutegravir-loaded lipid-based nanoparticles were successfully fabricated and optimised in the current research effort in order to minimise the drawbacks of conventional dosage form of dolutegravir and potentially improve the bioavailability and therapeutic result. For the preparation of lipid-based nanoparticles, Glyceryl monostearate is chosen as the solid lipid, Capmul MCM EP as the liquid lipid, Tween 80 and Span 80 as surfactants, PEG 6000 for PEGylation of lipid, propylene glycol as a drug solubilizer, and sodium cholate as a charge modifier. Under ideal circumstances, lipid-based nanocarriers demonstrated melt emulsification sonication technique entrapment effectiveness (88.09%) and smaller homogeneous particle size (155nm). The results suggest that DTG takes time to be released when encapsulated in the LNP because of its high solubility in liquid lipids, and the lipid layer is stabilised by glyceryl monostearate (a solid lipid), which contributes a major role in prolonging the release. In conclusion the optimized formulation is suitable for large scale manufacturing without the use of any organic solvents. In future, the improved lipid-based nano formulation will be able to produce dry powder that will go through freeze-drying processes and can be used for the treatment of HIV infections in paediatric patients.

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CONFLICTS OF INTEREST: The authors have reported no conflicts of interest regarding this investigation.

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