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Formulation and Evaluation of the Dolutegravir Nanocrystals

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Abstract:
Drugs with low water solubility may be delivered more easily with the help of nanocrystals. The use of low energy anti-solvent precipitation to create nanocrystals has been examined in this work, for the anti-retroviral medication dolutegravir, which is essentially poorly insoluble in water, stable nanocrystals with consistent particle sizes were created. Due to its limited water solubility, dolutegravir, a powerful HIV integrase inhibitor, has problems that impact both its bioavailability and effectiveness. The purpose of this study was to improve the solubility and bioavailability of dolutegravir by creating oral nanocrystal tablets. A bottom-up method was used to create the nanocrystals, and appropriate excipients were used to stabilize them. When compared to the pure medication, the optimized nanocrystal formulation showed better solubility and dissolution rates.The nanocrystal powder was used to produce oral tablets, which were then tested for pharmacokinetic profile and effectiveness in HIV-infected cells. The results demonstrated that the oral nanocrystal Dolutegravir tablets had better bioavailability and efficacy. This innovative formulation strategy has the potential to enhance HIV patients' treatment results.

INTRODUCTION:

Nanocrystals:

Currently, one of the primary uses of nanotechnology in medication delivery is to address the issue of hydrophobic medicines' low water solubility. Roughly 40% of all newly developed chemical entities are challenging to manufacture because they are poorly soluble in water[1]. Developing a highly effective medicine formulation is hampered by the molecule's poor solubility. Low solubility medications have irregular absorption and poor oral bioavailability, which is especially relevant for medications in class II of the Biopharmaceutical Classification System (BCS). [2] The oral bioavailability of a medicinal substance depends on its solubility and dissolution rate, which can impact treatment efficacy. To improve drug dissolution, various methods can be employed, including the anti-solvent method. This technique involves dissolving the drug in a solvent and then adding an anti-solvent that reduces the

drug's solubility, causing it to precipitate out of solution.[3] The anti-solvent method is a promising approach for enhancing the solubility and bioavailability of poorly water-soluble drugs, such as Dolutegravir, which falls under BCS Class II due to its low solubility and high permeability. By utilizing the anti-solvent method, researchers can produce nanoparticles or nanocrystals with improved solubility and dissolution rates.Benefits of Anti-Solvent Method : [4]

1. Improved solubility: Enhanced dissolution rate and solubility of poorly soluble drugs.
2. Increased bioavailability: Better absorption and therapeutic efficacy.
3. Nanoparticle formation: Production of nanoparticles or nanocrystals with improved properties.
4. Enhanced wettability: Improved interaction between the drug and the surrounding environment.

Dolutegravir, an antiretroviral medication for treating HIV-1 infection, can benefit from the anti-solvent method by improving its solubility and dissolution rate, researchers can potentially enhance its therapeutic efficacy and treatment outcomes.[5] A second-generation integrase strand transfer inhibitor called dolutegravir (DTG) prevents the HIV virus's DNA from integrating into host DNA, which is an essential step in viral reproduction. The ability of DTG to trigger enzyme-linked cations, which restricts viral DNA insertion in the host gene, is the hypothesized mechanism of action. Due to its comparatively lower level of body resistance, the DTG is recommended above other integrase inhibitors. 34% of it is bioavailable. It has a 14-hour half-life. DTG is classified as a drug under System II of the Biopharmaceutical Classification. The medications in BCS II exhibit high permeability and limited water solubility [4,6,7,8,9,10].

Advantages of Anti-Solvent Method over Other Techniques : The anti-solvent method offers several advantages, including:

1. Simple and cost-effective: Compared to other techniques, the anti-solvent method can be more straightforward and cost-effective.
2. Scalability: The anti-solvent method can be easily scaled up for industrial production.
3. Flexibility: The technique can be applied to various drugs and solvents.

By leveraging the anti-solvent method, researchers can develop more effective treatments for poorly soluble drugs like Dolutegravir.[10]

Composition of Nanocrystals: The nanocrystals are consists of different components but for the Anti-solvent method the following composition is used :

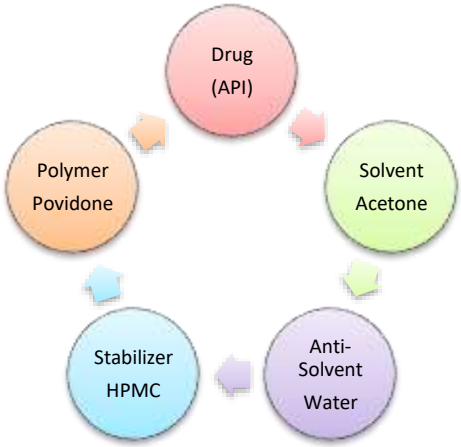

Figure 1 : The Composition of Nanocrystals

Defination of Nanocrystals : Surface-stabilized crystalline nanoparticles between 200 and 500 nm in size are called nanocrystals. By raising the saturation solubility, dissolution rate, and possibly

mucoadhesion, they enhance the oral bioavailability of medications with dissolution rate dependent bioavailability.[11]

MATERIAL AND METHOD

MATERIAL:

Dolutgravir was obtained as a gift sample from the (Hetro Lab Suraram,Hydrabad,Telangana) , Acetone (Amepurva Forums Nirant Institute of Pharmacy),Hydroxypropylmethylcellulose (Amepurva Forums Nirant Institute of Pharmacy),Povidone (Amepurva Forums Nirant Institute of Pharmacy).

METHOD:

PREPARATION OF NANOCRYSTALS :

Nanocrystals are prepared by using anti-solvent method.This method mainly consist two steps,prepration of solution one and preparation of solution two.To prepare the Solution 1: Drug is dissolved in the suitable organic solvent ,in which drug is very soluble.Now prepare the solution 2: Water is taken as the anti-solvent and the stabilizer is added .Latter is placed on magnetic stirrer at a constant speed of 1400 rpm for 30 min,like the way nanocrystals were prepared. Now inject the drug solution into the solution containing stabilizing agent with the help of syringe drop wise.Then it is stirred, centrifuged,and then suspended in distilled water and sonicated for 10min. After sonication it is filtered and dried to obtain Nanocrystals.

FORMULATION TABLE :

Ingredient	F1	F2	F3
Drug	1.5	1.5	1.5
Solvent	10	9	8
Anti-solvent	20	19	18
Stabilizer	0.7	0.6	0.5
Polymer	-	0.7	0.6

Table 1. Formulation Table.

EVALUATION OF NANOCRYSTALS:

Particle Size Analysis: Sample is diluted in the proportion of 1: 10 in methanol. Sonicated for 10 minutes and filtered through Whatman filter paper. Filtrate is taken for analysis. Light from the laser light source illuminates the sample in the cell. The scattered light signal is collected with detectors, at a 90 degree (right angle). Keep sample in cuvette. Go to condition set and fill name of sample, Run time for 120 seconds and select glass cuvette as sample holder. Start measurement and view the report in nanometers for particle size. Result will show value of Mean, Mode, median, SD, PD and z Average for particle size with graph contains mean value.

Entrapment Efficiency : The entrapment efficacy (EE) of nanocrystals dispersion was determined by the centrifugation method.The nanocrystals centrifuged at 2000rpm for one hour and then collected the supernatuant liquid of that dispersion. Then the collected liquid was filtered to measure the free drug concentration after making the dilution with freshly prepared phosphate buffer pH 7.4. The absorbance was measured at 266 nm.Following formula was used to calculation of entrapment efficiency.

Entrapment efficiency % = Amount of drug in NP (mg) / Amount of drug added (mg) × 100

Drug content : Take a known volume (e.g., 1 mL) of the prepared Nanocrystals formulation. Dissolve the Nanocrystals by adding an appropriate solvent (e.g., methanol) & filter the solution. Use a UV spectrophotometer to measure the concentration of the drug the solution. Set the wavelength range 400nm to 200nm & check the absorbance at 266nm. & Calculate the total drug content using Beer's Law.

In vitro drug release : In vitro drug release of Dolutegravir Nanocrystals was determined using type II Apparatus of IP(Basket). The cellophane membrane was mounted between the donor and receptor compartments. The receptor compartment was filled with phosphate buffer (pH 7.4) at 37°C. The solution was stirred at 75 rpm. The Dolutegravir Nanocrystals was placed on cellophane membrane and the compartments were clamped together. One ml of sample was withdrawn at predetermined time for 1.5hours, from receptor compartments and immediately replaced using phosphate buffer after filtering through 0.45µm filter and appropriate dilutions, the sample were analysed for drug content at 318nm.

RESULT:

PREFORMULATION STUDY :

FTIR :

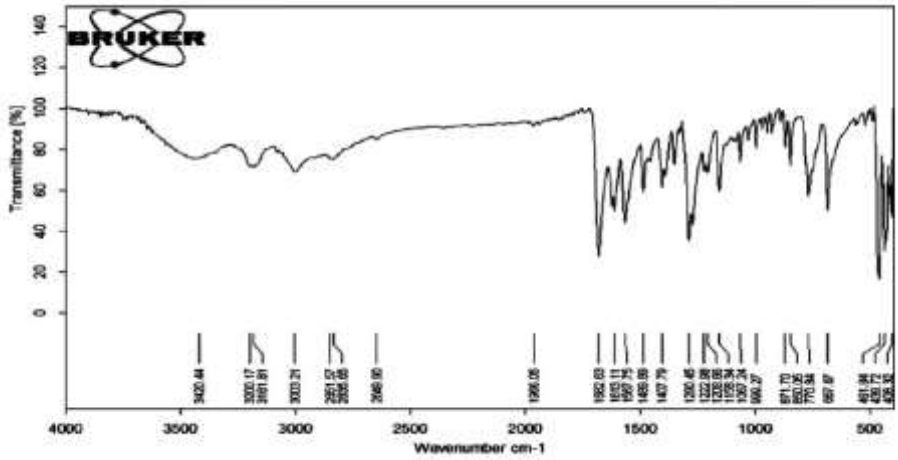

Figure 2: Systematic Representation of FTIR of Dolutegravir.

SR.NO	REPORTED RANGE	OBSERVED RANGE	FUNCTIONAL GROUP
1	(3550-3200 cm^{-1})	3344.57 cm^{-1}	O-H stretch (Alcohol)
2	(3000-2800 cm^{-1})	2917.62 cm^{-1}	C-H stretch(Alkane)
3	(1725-1705 cm^{-1})	1711.32 cm^{-1}	C=O stretch(Carbonyl)
4	(1650-1550 cm^{-1})	1631.45 cm^{-1}	C=C stretch (Aromatic Ring)
5	(1250-1020 cm^{-1})	1150.60 cm^{-1}	C-N stretch(Amine)
6	(1200-1100 cm^{-1})	1150.50 cm^{-1}	S=O stretch (Sulfonateor Sulfate)

Table 2 : Interpretation of FTIR of Dolutegravir .

NANOCRYSTALS EVALUATION :

PARTICLE SIZE

The nanoparticle size of dolutegravir created using the antisolvent method generally falls within the range of 100 nm to 455 nm. Researchers have observed particle sizes as low as 75.3 nm in optimized formulations and as high as 455 nm in other studies. The exact size can vary depending on the specific formulation and experimental conditions.

The dolutegravir nanoparticles had a mean size of 337.1 nm, a low polydispersity index, and a negative zeta potential, indicating good stability.

PARAMETER	STANDARD VALUE	VALUE OBTAINED
Particle Size Range	10-1000 nm	100-455 nm
Mean Particle Size	100-500 nm	337.1 nm
Polydispersity Index	<0.5	<0.5
Zeta Potential	±20mV to ±30mV	-20 to -30
Distribution Form	Monodisperse	Monodisperse

Table 3. Measurement of particle size of Standard Values .

	F1	F2	F3
Scattering Angle	90˚	173˚	90˚
Dispersion Medium	0.896 mPa.s	0.897 mPa.s	0.897 mPa.s
Distribution Form	Monodisperse	Monodisperse	Monodisperse
Count Rate	2826 kCPS	1977 kCPS	2132 kCPS
Aspect Ratio	1.00	1.00	1.00
Mean	156.7 nm	167.8 nm	168.9 nm
Standard Deviation	35.3 nm	48.4 nm	39.8 nm
Mode	143.8 nm	160.2 nm	161.0 nm
Polydispersity Index	0.223	0.563	0.233
Z Average	159.8 nm	418.9 nm	161.3 nm

Table 4. Measurement of particle size .

ENTRAPMENT EFFICIENCY

BATCH	ENTRAPMENT EFFICIENCY
F1	92.50
F2	93.81
F3	91.16
F4	95.75

Table 5. % entrapment efficiency of formulation

The % entrapment efficiency of different batches of Dolutegravir nanocrystals prepared using the anti-solvent method. The values indicate that the nanocrystals have high entrapment efficiency, with batch F4 showing the highest value of 95.75%.

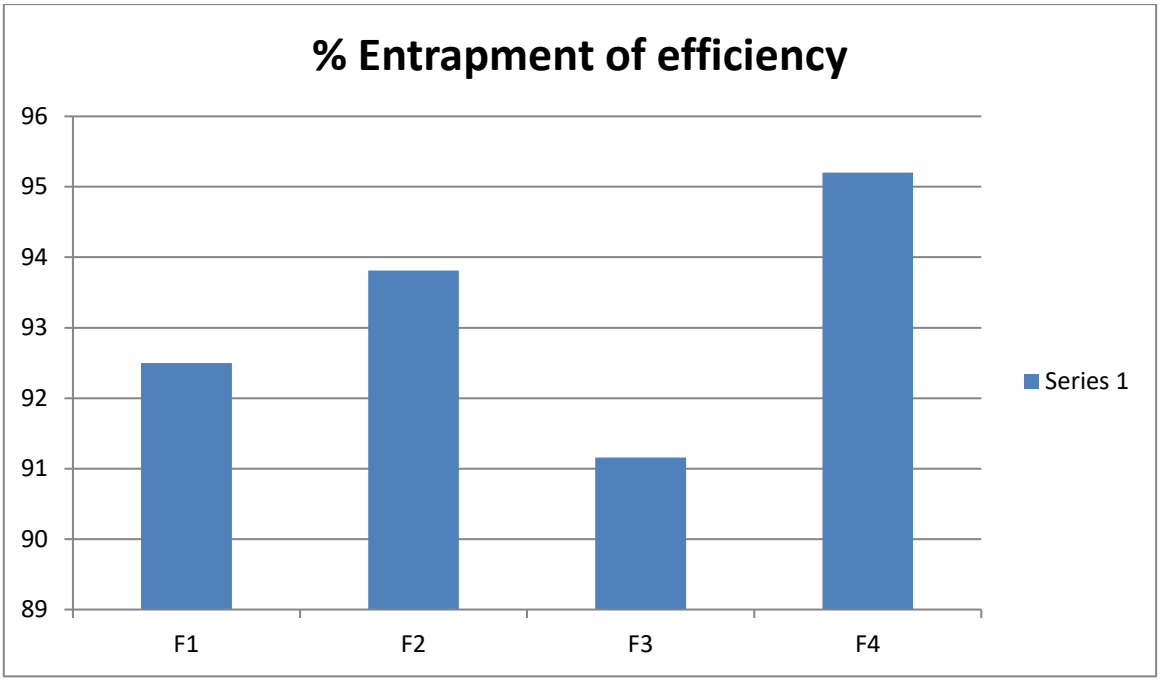

Figure 3 : Systematic Representation of % entrapment efficiency of Formulation

DRUG CONTENT

BATCH	% DRUG CONTENT
F1	95.20
F2	96.45
F3	93.48
F4	97.50

Table 6 : % of drug conent formulation batches

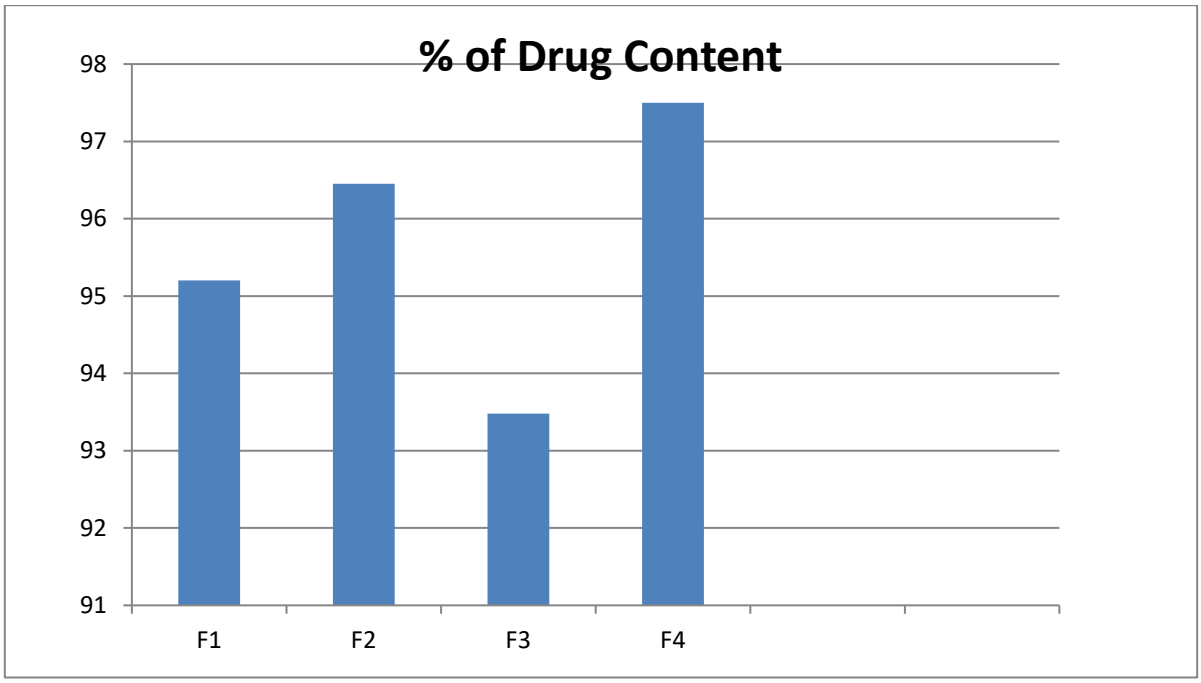

Figure 4: Systematic Representation % of Drug Content

This Figure 3 shows the % drug content of different batches of Dolutegravir nanocrystals prepared using the anti-solvent method. The values indicate that the nanocrystals have high drug content, with batch F5 showing the highest value of 98.10%.

DRUG RELEASE

TIME		% DRUG RELEASE	
	F2	F3	F4
30 min	26.55	25.35	25.95
60 min	60.15	57.60	60.00
90 min	95.40	91.95	95.48

Table 7. % of drug release of formulation batches.

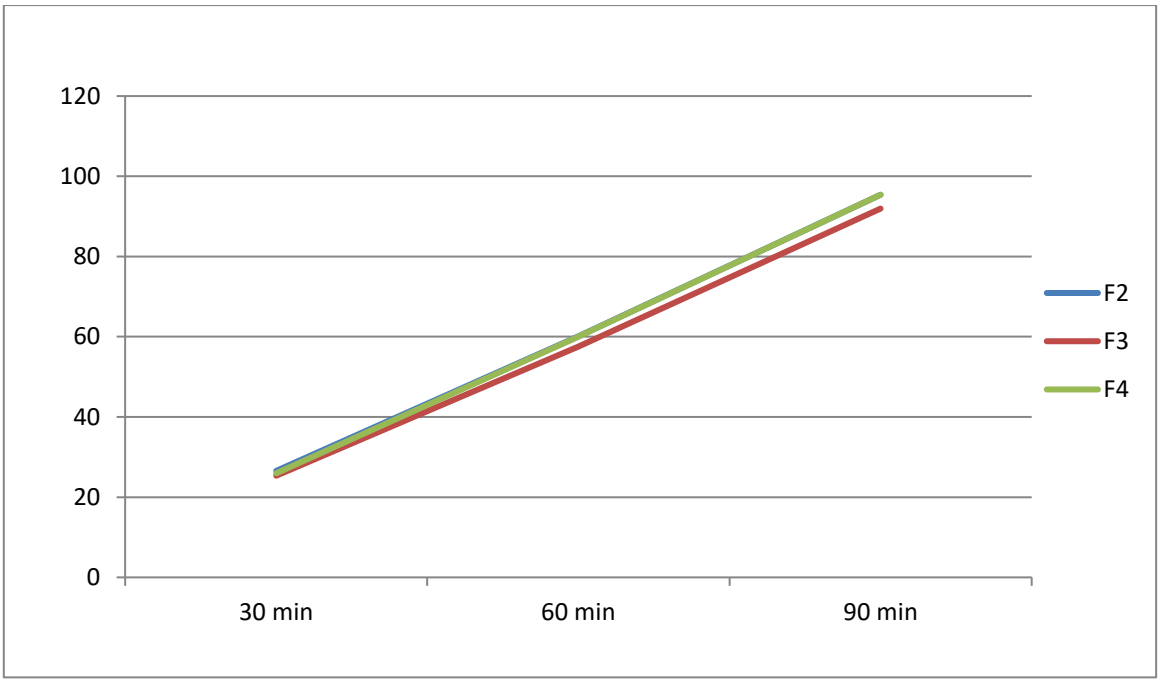

Figure 5 : Systematic Representation of % of Drug Release

This figure shows the % drug release of Dolutegravir nanocrystals prepared using the anti-solvent method at different time points. The values indicate that the nanocrystals exhibit sustained drug release, with a significant increase in drug release at 90 minutes.

Conclusion:

1. Particle Size : The particle size of all formulated batch have size range of nanometer.So all the batches pass the test.
2. The % entrapment efficiency of formulation batch of 2,3,4 are 93.81, 91.16, 95.75 respectively .
3. Drug Content : The % of drug content of formulation batches F2,F3,F4, shows the value within range are 96.45,93.48,97.50, respectively.
4. Drug Release : The drug release of formulation shows the study of release of drug with the R2 value of F2,F3,F4 are 25.95, 60.00, 95.40 respectively.

From all the Results and Discussion,of Formulation batches ,batch 4 is optimum batch..

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