FORMULATION DEVELOPMENT AND IN VITRO EVALUATION OF THE RITONAVIR NANOSUSPENSION BY USING ANTISOLVENT PRECIPITATION-ULTRASONICATION METHOD

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Article History: Received: 12.10.2022 **Revised:** 20.11.2022 **Accepted:** 22.12.2022

Abstract:

Ritonavir, a widely prescribed antiretroviral drug, belongs to BCS class II and due to its poor water solubility, its oral bioavailability is low. The present focuses on the Formulation development and in vitro evaluation of the Ritonavir Nanosuspension by using antisolvent precipitation-ultrasonication method. for the formulation of the nanosuspension suitable polymer concentration and solvent and anti-solvent, string time and sonication time was selected based on the preliminary studies, the optimized formulation was selected based on the mean particle size was 287.3 nM, and the PDI was 0.385. The zeta potential was noticed 26.75 ± 3.25 mV, The dissolution data of the optimized formulation was done and studies shows release rate at 120 min shows at 98.2 %. The Accelerated stability studies shows that Mean particle size (nM) ranging from 241.1 to 282 nM , Saturation solubility (µg/ml) ranging from 107.85 to 111.85, CPR at 2 min (% w/w) ranging from 94.12 to 97.90 and Drug content (%w/w) ranging 98.52 to 102.12 respectively. From the regression values, it was determined that the optimized formulation exhibits zero-order drug release. The determined n value of 0.84 indicates that drug release is characterized by non-Fickian transport.

Keywords: Ritonavir, Nanosuspension, Drug content, antisolvent precipitation-ultrasonication method

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DOI: 10.53555/ecb/2022.11.12.239

1. INTRODUCTION

A dosage form's bioavailability is its most crucial attribute. To achieve the intended pharmacological effect, the dosage form must be able to transport the active component to the site of action. A drug's bioavailability can be defined more accurately as the pace and extent to which it is absorbed into the systemic circulation from its dose form. Many factors related to the medicine, its formulation, and the patient affect this. Particle size, chemical form, drug solubility, formulation and production variables (excipient type and quantity, compression pressure, etc.), and dose form are all factors that can significantly affect medication bioavailability. A drug's low water solubility and dissolving rate are well-known to significantly restrict its bioavailability and effectiveness. In order for the digestive system to absorb medicine in solid dosage form (tablets), the medicine must dissolve first. When a medicine is not very soluble in water, dissolution becomes the most important phase in the absorption process. [1].

Many current medications are BCS Class II, which has low solubility and high permeability. Low and variable bioavailability and solubility characterize these medications, and they are insoluble in water and aqueous solutions within the pH range of 1.0 to 7.5. There is an urgent need to find ways to increase the bioavailability and dissolving rate of these "BCS" Class II medications. One of the biggest challenges in medication development is improving poorly soluble drug dissolution and oral bioavailability. [2]

Ritonavir, a widely prescribed antiretroviral drug, belongs to BCS class II and due to its poor water solubility, its oral bioavailability is low and variable. Ritonavir is almost insoluble in water and aqueous solutions. Its solubility in water6 has been reported to be 2.56 mg/100 mL. Thus, oral absorption of ritonavir is limited by dissolution rate and requires improvement of solubility and dissolution rate to increase its oral bioavailability.it is chemically called as 5-thiazolylmethyl{(αS)-α-[(1S,3S)-1-hydroxy-3-((2S)-2-{3-[(2-isopropyl-4-thiazolyl) methyl] methyl ureido} 3-methylbutyramido)-4-phenylbutyl]phenethyl}carbamate [2-5].

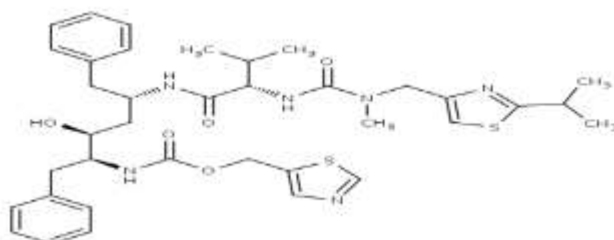

Figure1: Chemical structure of Ritonavir

Experimental work:

2. MATERIALS AND METHODOLOGY

Ritonavir API Sample was collected from the Hyderabad chemical drug agency, Hyderabad, The Excipients like Poloxamer 188, Poloxamer407, Ployvinyl alcohol, PVP K30, Sodium laurel sulphate, Dichloromethane, Ethanol, Methyl

alcohol, 2-Propanol, Butanol, Ethyl Acetate, Dimethyl sulphoxide, Mannitol AR grade were purchased and collect from Finnar Chemicals, Ahmedabad. All the instruments used in the work were calibrated.

Methodology: [6]

Preformation studies:

Melting point by capillary tube method

The melting point of ritonavir was determined by the capillary tube method. A sufficient amount of ritonavir powder was introduced into the capillary tube, resulting in a compact column of 4-6 mm height. The tube was placed in an electric melting point apparatus and the temperature was raised. The melting point, which is the temperature at which the last solid particle of ritonavir in the tube entered the liquid phase, was recorded.

Drug and excipient compatibility studies

The drugs and excipients selected for the formulation were evaluated based on physical and chemical compatibility studies.

Physical compatibility study

Physical compatibility studies have been conducted to provide valuable information to the formulator in the selection of appropriate excipients for the formulation. It was prepared by mixing drugs and excipients and placed at room temperature, 40 °C and 75% relative humidity. All color changes in the physical mixture were observed visually.

Solubility studies of pure ritonavir

A solubility analysis of the preformulation was performed, which includes the selection of a suitable solvent to dissolve the respective drug. Solubility was determined by adding the solute in small portions to a fixed volume of solvents, shaking the system vigorously after each addition and visually inspecting for undissolved solute particles. If a certain amount of solute remains insoluble, the total added up to that point was a good and quick estimate of solubility.

Determination of the absorption maximum (λ max) of Ritonavir

The stock solution is prepared with 50 mg of Ritonavir which has been accurately weighed and transferred to a 50 ml volumetric flask. The drug was dissolved in 5 ml of acetone and the volume was adjusted to 50 ml with pH-phosphate buffer 6.8 to obtain a 1000 µg/ml stock solution. 1 ml of this stock solution was re-diluted with phosphate buffer pH 6.8 to 10 ml to obtain a 100 µg/ml solution. The resulting solution was scanned with a UV-visible spectrophotometer at a wavelength of 200-400 nm to obtain the absorption maximum λmax.

Scanning and calibration curve preparation of ritonavir

For estimation of drug content in ritonavir nanosuspension, a calibration curve was prepared in methanol as ritonavir is having a solubility in methanol.

Scanning and calibration curve preparation of ritonavir in methanol

Ritonavir standard solution (100 µg/ml) was prepared by accurately weighing 10 mg of ritonavir and dissolved in 5 ml of methanol in a 100 ml volumetric flask. It was sonicated for 5 minutes and made up with 100 mL of methanol. A solution of ritonavir (10 µg/ml) in methanol was prepared from the standard stock solution and UV scanning was performed in the wavelength range of 200 to 400 nm and the wavelength showing the maximum absorbance was selected as λmax for further analysis.

Appropriate aliquots of the standard stock solution (100 µg/ml) were transferred to different 10 ml volumetric flasks and made up to 10 ml with methanol to obtain a concentration of 5–30 µg/ml. The absorbance of these solutions was measured at the chosen λmax. The experiment was performed in triplicate to

confirm the calibration curve. A calibration curve was generated

Selection of solvent and antisolvent

The solubility of several medicines in various solvents and their combinations was investigated. The drug's solubility in the various solvents was used to select favorable and poor solvents. In particular gravity bottles, 10 mg of medication was mixed with 10 ml of solvent. This amount was enough to create a saturated solution. By keeping these specific gravity bottles in a cryostatic constant temperature reciprocating shaker bath, they were shaken at 100 RPM for 24 hours at 25°C. The bottles were then opened, and the solutions were filtered using Whatman filter paper (0.22 m). The absorbance of the solution was measured at the medicines' respective maximums. This procedure was performed three times.

Preparation of nanosuspension by antisolvent precipitation-ultrasonication method [7-8]

Nanosuspension was ready by the precipitation-ultrasonication strategy. The medication was broken up in methanol by sonication for 5 min at room temperature. Various stabilizers were disintegrated in water to get a progression of antisolvent. The two arrangements were gone through a 0.45µm filter. The antisolvent was cooled to 3°C in an ice-water shower. Then, at that point, drug arrangement was immediately presented through a needle situated with the needle straightforwardly into stabilizer arrangement into 40 ml of the pre-cooled antisolvent at various mixing speed under above stirrer to permit the unstable dissolvable to vanish at room temperature for 3-4 hours. After precipitation of antisolvent, the example was quickly moved to a test tube and was treated with a ultrasonic test at various time lengths (in mins). The test with a tip breadth of 6 mm was submerged in the fluid, bringing about the wave voyaging downwards and reflecting upwards. Cluster size for readiness of nanosuspension was taken 40 ml.

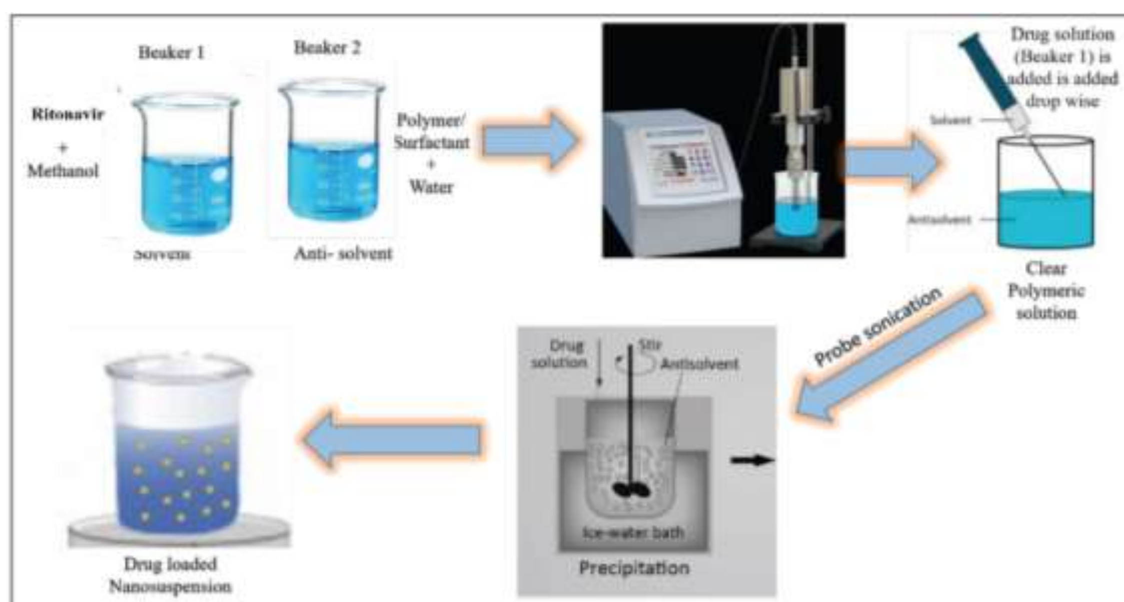

Figure 2 Formulation of nanosuspensions by solvent and anti-solvent Technique.

Lyophilization of nanosuspension of optimized batch

The nanosuspension was lyophilized and turned into dry powder using mannitol (1:1, Total solid: Cryoprotectant) as a cryoprotectant. During the lyophilization procedure, the sample was kept in the chamber and the temperature was kept at -80°C for 8 hours. After 6-8 hours, the nanosuspension was turned into dry powder and removed from the chamber, where it was stored in an airtight container for future use.

Selection of stabilizer

Various stabilizers like Polyvinyl Liquor, PVP K-30, Sodium Lauryl Sulfate, Poloxamer 188 and Poloxamer 407 were screened by planning nanosuspensions and estimating their immersion dissolvability, mean molecule size, polydispersity record (PDI) and zeta potential for determination of all that one which can be used for additional exploration work

Drug-excipient compatibility study

Investigations of medication excipient similarity address a significant stage in the preformulation phase of the advancement of all dose structures. The expected physical and compound cooperations among drugs and excipients can influence the substance, physical, restorative properties and soundness of the measurement structure. FTIR and DSC study were chosen for checking of medication excipient similarity study.

Fourier transformed infrared (FTIR) spectroscopy

FTIR spectroscopy was directed utilizing a Shimadzu FTIR 8400 Spectrophotometer (Shimadzu, Tokyo, Japan) and the range was kept in the frequency district of 4000-400 cm-1. The technique comprised of scattering test (drug, stabilizer, actual combination) by KBr pellet strategy. The pellet was set in the light way and the range was recorded.

Differential scanning calorimetry (DSC)

DSC was performed utilizing DSC-60 (Shimadzu, Tokyo, Japan) calorimeter to concentrate on the warm way of behaving of the example (drug, stabilizer, actual combination and lyophilized nanosuspension). The instrument contained the calorimeter (DSC 60), stream regulator (FCL60), the warm analyzer (TA 60WS) and working programming (TA 60). The examples were warmed in airtight fixed aluminum skillet under air environment at a checking pace of 10°C/min from 30°C to 330°C in an air climate. Void aluminum container was utilized as a kind of perspective.

Evaluation of nanosuspensions [9-10]

Particle size and PDI

Mean molecule endlessly size dissemination (polydispersity file) of the pre-arranged still up in the air by utilizing Zetasizer [Zetatrac, Microtrac, Japan] which follows the standard of light diffraction, additionally called Photon Connection Spectroscopy (computers). Preceding the estimation, the examples were properly weakened with water to an appropriate dissipating force and yet again scattered by shaking before measurement

Zeta potential

The Zeta potential is a proportion of the electric charge at the outer layer of the particles, demonstrating the actual dependability of colloidal frameworks. The zeta potential qualities higher than |30mV| show long haul electrostatic security of watery scatterings. In this review, the Zeta Potential was surveyed by deciding the electrophoretic portability of the particles utilizing Zetasizer [Zetatrac, Microtrac, Japan]

Drug content

An aliquot (1ml) of the pre-arranged nanosuspension was weakened in methanol and separated with a 0.2 µm channel. All out drug still up in the air by

UV spectrophotometer at λmax of the medication.

Saturation solubility
The immersion dissolvability of arranged nanosuspension was performed by filling it in a vial and saved for 48 h mixing with the assistance of attractive stirrer at 100 RPM to guarantee immersion. Then 2 ml of nanosuspension was filled in an eppendorf tube and centrifuged at 10,000 RPM for 30 minutes. The supernatant was separated through a 0.2µm needle channel and examined by UV-noticeable spectrophotometer [UV-1800, Shimadzu, Japan] at λmax of the medication after reasonable weakening with disintegration media which was utilized as a clear. Three-fold examination of each example was completed. By utilizing the adjustment bend, immersion solvency was determined.

n-vitro dissolution study
An in-vitro disintegration study was performed utilizing USP 24 oar instrument (ELECTROLAB TDT-06P). The disintegration medium was taken according to Table 4.3. To limit frothing of the medium during the analysis, the medium was delicately moved into the disintegration vessel. Disintegration was performed at 37ºC, utilizing an oar speed determined in following Table 4.3. Nanosuspension comparable to a portion of the medication was added to the disintegration vessels. 5 ml tests were removed at a particular time 10,20,30,40,50,60,70,80,90,100,110,120 mins and sifted quickly through 0.2 µm needle channel and dissected spectrophotometrically. Consequently, 5 ml of new medium was added to the disintegration vessel . The trials were acted in three-fold and the mean qualities were accounted for.

Dissolution Condition	Ritonavir Nanosuspension
Dissolution media	0.05M Phosphate buffer pH 6.5
Volume of Dissolution media	250
Speed in RPM	50
Sampling Intervals	10,20,30,40,50,60,70,80,90,100,110,120 mins
Dose of drug	100 mg

Table 4: Dissolution study

Scanning electron microscopy (SEM)
Scanning electron microscopy (EVO-18, ZEISS, Germany) was used to examine the surface properties of lyophilized nanosuspensions from 3kx to 28kx. The samples were mounted on double-sided carbon adhesive tape that had previously been attached on brass stubs and then gold coated by sputter coater for 4 minutes at a process current of 10 mA. 15 kV was the accelerating voltage.

Accelerated Stability study as per ICH Guidelines
According to ICH recommendations, accelerated stability experiments of lyophilized nanosuspension were undertaken at 25±2°C and 60±5% RH for 6 months. The nanosuspension was

lyophilized and encased in firm gelatin capsules. The samples were extracted at regular intervals (0, 1, 3, and 6 months) and analysed for particle size, saturation solubility, % CPR at 2 min, and % w/w of drug concentration.[11].

Kinetics Study of optimized formulation [12]

The amount of drug released from ritonavir Nanosuspension was determined by calculating the total value of each point in time using a flexible model. The drug kinetics release mechanism accomplished from hydrophilic matrices, as well as the dissolving outcome of the corresponding nanosuspension batch, were processed using several kinetic equations, including zero and first-order kinetics, Higuchi, Hixson Crowell, and Korsmeyer-Peppas models.

To determine the kinetics of release, data from in vitro drug release readings were displayed in various kinetic models.

1)**Zero order kinetic– Cumulative % drugs released versus Time.**

$Q = Q0 + kt$ ………. (1)

Where, Q = initial concentration of drug at time t = 0

Q0 = amount of drug dissolved at a time t

k = zero order constant in concentration/time

t = time in an 'h'

2)First order kinetic–Log cumulative percent drug remaining versus Time.

$\text{Log } Q = \text{Log } Q0 – Kt/ 2.303$ ……… (2)

Where,

Q = % amount of drug dissolved at time t,

Q0 = initial amount of drug dissolved at t=0,

k = first order rate constant

t = time in hrs

3) Higuchi's model– Cumulative percent drug released versus square root of Time.

$Qt = Kt^{1/2}$ ……….. (3)

Where,

Qt = amount of drug released at a time't' per unit area A

K = constant reflecting design variable system (differential rate constant)

t = time in hrs.

4) Hixson Crowell model- Cube root of log cumulative percentage of drug remaining vs. log Time.

$Q0^{1/3} – Qt^{1/3} = Kt$ ……….. (4)

Where,

Q0 = initial amount of drug in the pharmaceutical dosage form,

Qt = remaining amount of drug in the pharmaceutical dosage form at time t

K = Hixson-Crowell proportionality consistent

5) Korsmeyer equation–Log cumulative percent drug released versus log Time.

Based on the highest regression values for correlation coefficients for formulations, the best- fit model was decided.

$Mt / M\alpha = Kt^n$ ……….. (5)

Where,

Mt =Fraction of drug released at time t

Mα = amount released at a time t = α

K = kinetics constant (instructing structural and geometric characteristic of the formulation)

n = Diffusion exponent

The value of 'n' in this model characterises the drug release mechanism, as detailed in the table below.

As illustrated in Table 5, a plot of log (drug release) vs log t will be linear with a slope of n and an intercept that gives the value of log k. To investigate the release kinetics, data from in vitro drug release trials were displayed as log cumulative percentage drug release vs. log time.

S.No.	Diffusion exponent value (n)	Drug release mechanism
1	< 0.45	Fickian release
2	0.45 to 0.89	non-Fickian transport
3	0.89	Case II transport
4	> 0.89	Super case II transport

Table 5: Diffusion exponent values indicating drug release mechanism

3. RESULTS AND DISCUSSION

Scanning and calibration curve preparation

Scanning and calibration curve preparation of ritonavir in methanol

The standard stock solution of ritonavir was produced in methanol according to the technique stated in the experimental section and scanned between 200 and 400 nm using a UV Visible spectrophotometer. Figure 3 depicts the UV absorption spectrum of ritonavir, which showed a maximum at 254 nm.

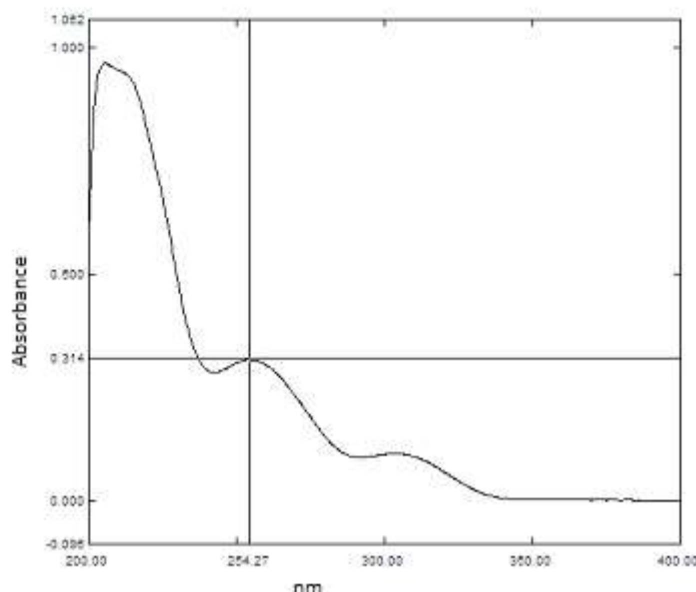

Figure 3 : Scanning curve of ritonavir in methanol

A calibration curve in methanol in the range of 5-30 g/ml was created using a UV-Visible spectrophotometer. These solutions' absorbance was measured at 254 nm. To validate the calibration curve, this method was repeated three times. The values were tabulated in Table 6

Sr. No.	Concentration (µg/ml)	Absorbance at 254 nm* (Mean ±SD)
1	0	0.000±0.00

2	5	0.172 ±0.12
3	10	0.332±0.22
4	15	0.475±014
5	20	0.622±0.11
6	25	0.775±0.05

Table 6: calibration data of ritonavir in methanol

*Indicates average of three determinations

As illustrated in Figure 6, a calibration curve was created by graphing absorbance vs. concentration in g/ml, and a regression equation was found to be Y = 0.0303X + 0.0147 with a regression coefficient of 0.9995.

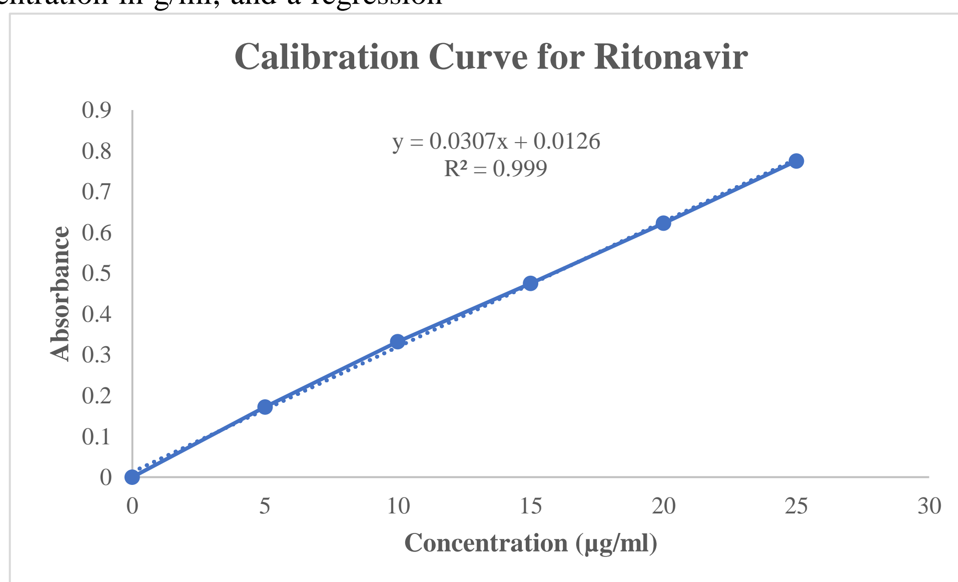

Figure 4: Calibration curve of ritonavir in methanol

Selection of solvent and antisolvent

The solvent and antisolvent were chosen based on the solubility of ritonavir in several solvents and their combinations. Because the medication had the maximum solubility (6.12 mg/ml) in methanol and the lowest solubility (0.0014 mg/ml) in water, they were chosen as solvent and antisolvent, as shown in Table 7

Drug	Solvents	Solubility (mg/ml) (Mean ± SD) *
	Water	**0.00125 ±0.0011**
	Methanol	**6.12 ± 0.48**
	Alcohol	3.32± 0.21

Ritonavir	Iso-propanol	0.321 ±.00134
	N-Butanol	0.125 ± 0.325
	Alcohol:2-Propanol (1:1)	0.65 ± 0.225
	Alcohol: Butanol (1:1)	0.145 ± 0.321
	Ethyl Acetate	0.354 ± 0.321

Table 7: Result of Selection of solvent

*Indicates average of three determinations

Selection of stabilizer

Polyvinyl alcohol, PVP K-30, sodium lauryl sulphate, poloxamer 188, and poloxamer 407 were screened by producing nanosuspensions with the formulation and processing parameters listed in Table 8

Batch Code	Stabilizers	Stabilizers Concentration (mg/ml)	Amount of Ritonavir(mg)	Stirring Speed (RPM)	Stirring Time (h)	Sonication Time (min)	Solvent: Antisolvent Volume Ratio
RF1	Polyvinyl Alcohol	40	100	1000	4	20	1:20
RF2	PVP K30	40					
RF3	Sodium Lauryl Sulphate	40					
RF4	Poloxamer 188	40					
RF5	Poloxamer 407	40					

Table 8: formulation and processing parameters for selection of stabilizer

*Indicates average of three determination

As indicated in Table 9, the produced nanosuspensions (RF1 to RF5) were evaluated by evaluating their saturation solubility, mean particle size, polydispersity index (PDI), and zeta potential in order to pick the optimum stabiliser for future research.

Formulation Code	Stabilizer used	Saturation Solubility (µg/ml) (Mean ± SD) *	Mean Particle Size (nM) (Mean ± SD) *	PDI ((Mean ± SD) *	Zeta Potential ((Mean ± SD) *)
RF1	Poly Vinyl Alcohol	48.25 ±0.23	582.2 ± 2.5	1.023 ±0.23	-32.15 ± 1.02
RF2	**PVP K-30**	**82.12 ±0.31**	**252.1± 3.2**	**0.295± 0.32**	**-33.12 ± 0.23**

RF3	Sodium Lauryl Sulphate	53.12 ±0.98	423.2 ±1.5	0.735 ± 0.21	-30.17 ± 0.23
RF4	Poloxamer 188	62.32 ±0.53	375.2± 2.1	0.551 ± 0.21	-31.45 ± 0.25
RF5	Poloxamer 407	58.12± 0.12	589.5± 2.3	1.089 ± 0.24	-21.25 ± 1.24

Table 9: evaluation results of the stabilizer used

*Indicates average of three determinations
It was found that the PVP K-30 has the smallest mean particle size and the highest saturation solubility. Moreover, it possessed the lowest PDI, which signified homogeneity in nanosuspension particle size, and the highest zeta potential, which indicated greater stability. As a result, PVP K-30 was selected as a stabilizer to conduct further research.

S.No	**Ingredients**	**RF-01**	**RF-02**	**RF-03**	**RF-04**	**RF-05**	**RF-06**	**RF-07**	**RF-08**	**RF-09**
1	Ritonavir (mg)	100	100	100	100	100	100	100	100	100
2	PVP K30 (mg/ml)	10	15	20	25	30	35	40	45	50
3	Pluronic F-68	10	10	-	-	-	10	10	10	10
4	Methanol	10	10	10	10	10	10	10	10	10
5	Water up to	100	100	100	100	100	100	100	100	100

Table 10 Formulation design of Ritonavir nanosuspension using solvent and anti-solvent Method

Drug-excipient compatibility study
Conducting preformulation studies to assess the compatibility of drugs and excipients is an essential step in the development of all types of dosage forms. Potential physical and chemical interactions between medications and excipients can impact the chemical, physical, therapeutic properties, and stability of the dosage form. The methods selected for drug-excipient compatibility testing were Fourier transformed infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC).

Fourier transformed infrared (FTIR) spectroscopy
The spectra was obtained in the wavelength band 4000-400 cm-1 using a Shimadzu FTIR 8400 spectrophotometer (Shimadzu, Tokyo, Japan), figure 5 (A),5 (B),5 (C).

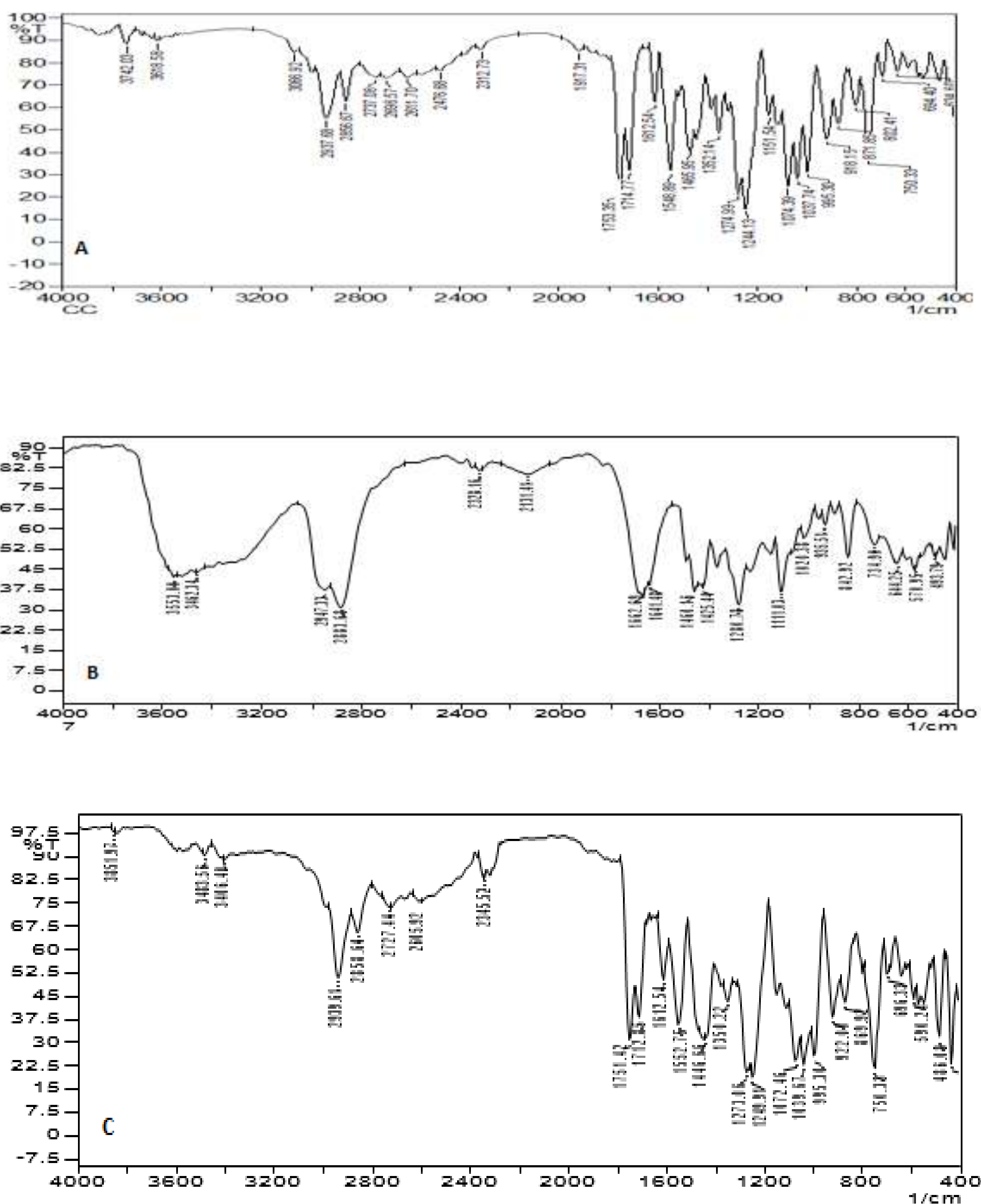

Figure 5 (A) Ritonavir, (B) PVP K-30, (C) Mixture of Ritonavir and PVP K-30

Differential scanning calorimetry (DSC)

The thermal behavior of samples (ritonavir, PVP K-30, physical mixture, and lyophilized nanosuspension) was assessed using a DSC-60 (Shimadzu, Tokyo, Japan) calorimeter. Figure 6 illustrates the outcomes of a DSC thermal analysis.

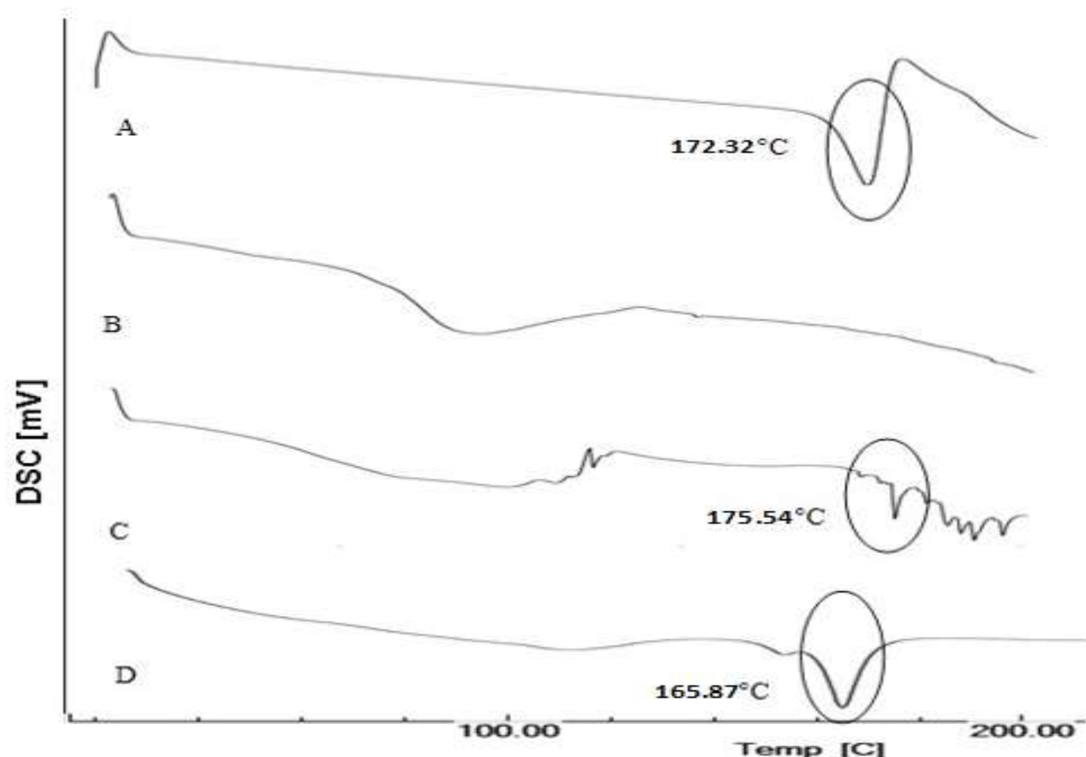

Figure6: (a) ritonavir, (b) PVP K-30, (C) physical mixture, and (d) lyophilized nanosuspension

In DSC experiments, ritonavir had a melting endotherm of 172.32°C, while lyophilized nanosuspension had a melting endotherm of 165.87°C. The decrease in the melting endotherm of ritonavir nanosuspension could be attributed to particle size reduction via antisolvent precipitation followed by ultrasonication.

Identification of optimized formulation with the PVP K-30

Ritonavir nanosuspension was made utilizing PVP K-30 as a stabilizing agent. Three distinct quantities of PVP K-30, such as 30 mg, 40 mg, and 50 mg were chosen. Nanosuspensions were made using the procedure outlined in the experimental section. The nanosuspensions that were generated underwent evaluation using several criteria, including the average size of the particles and saturation solubility. This evaluation was conducted in order to determine the appropriate amount of PVP K-30 for subsequent formulation work. The agitation speed is a major processing parameter in the creation of nanosuspensions 1200 RPM were selected to optimize the mixing velocity. a sonication time of 30 minutes was selected since it resulted in the smallest average particle size and the highest solubility at saturation.

Formulation Code	**Mean Particle Size (nm) (Mean ± SD)**	**Saturation Time (µg/ml)**
RF-01	325.12 ± 0.15	75.023 ± 1.25

RF-02	425. 05±0.48	79.25 ± 2.12
RF-03	412.32 ± 0.15	81.25 ± 1.11
RF-04	495.01 ± 0.35	87.15 ± 1.15
RF-05	325. 05 ± 1.22	85.12 ± 2.15
RF-06	425.12 ± 0.21	87.52 ± 1.24
RF-07	335 ±0.23	88.54 ± 1.12
RF-08	312.1± 2.2	91.89 ± 1.24
RF-09	**285.14 ±0.23**	**95.14 ± 1.14**

Table:11 Results of optimized preliminary parameters of the Ritonavir Nanosuspension

*Indicates average of three determinations
Based on the results of the formulations RF-01 to RF-09 conclude that, the RF-09 evaluation results shows that the Mean Particle Size 285.14 ±0.23 nm Saturation Time 95.14 ± 1.14 µg/ml in remaining formulations. The decrease the particle size of RF-09 indicate the is is optimized formulations

Evaluation of optimized batch of Ritonavir Nanosuspension (RF-09)

Particle size and PDI

The optimized batch's mean particle size was 287.3 nM, and the PDI was 0.385. The final formulation of ritonavir nanosuspension was designed for oral administration, where PDI and particle size greater than 5m are not necessary. A nanosuspension's average particle size ranges between 200 and 1000 nm.

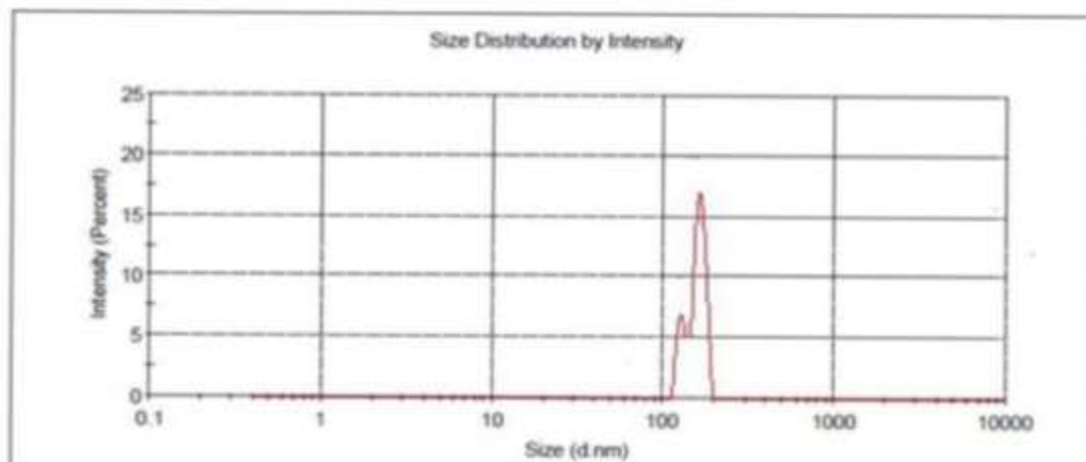

Figure 9 Particle size and PDI graph of the optimized formulation

Zeta potential

PVP K-30 is a notable effective polymeric stabilizer framing adsorption layers of medication nanoparticles by and large, zeta expected worth of ± 30mV is adequate for the solidness of nanosuspension. Zeta capability of improved detailing was noticed 26.75 ± 3.25 mV which follows the prerequisite of zeta potential.

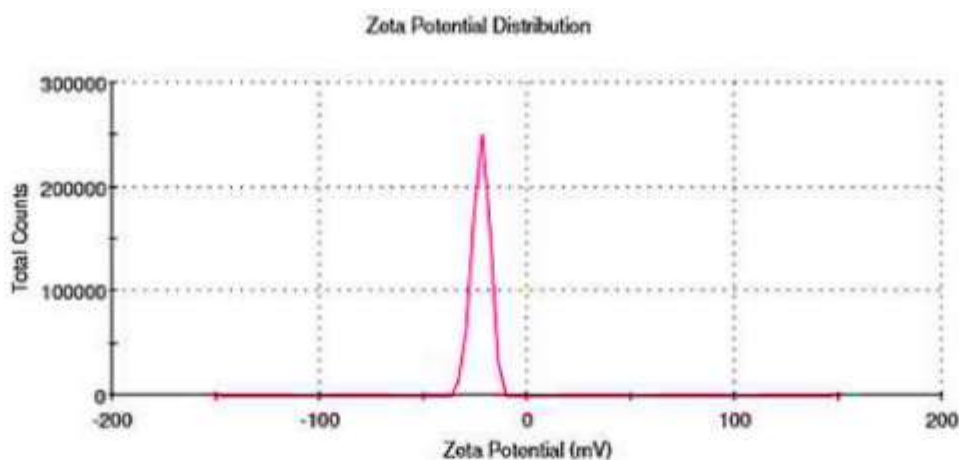

Figure 10 Zeta potential graph of the optimized formulation RF-09

Drug content:
An aliquot (1 ml) of the pre-arranged nanosuspension was weakened in methanol and separated with a 0.2 μm channel. Complete medication not set in stone by UV-Apparent spectrophotometer at 254 nm and was viewed as 99.98 % w/w of the ritonavir.

Immersion dissolvability
Immersion dissolvability of a streamlined cluster of ritonavir nanosuspension was found to be 109.8 μg/ml. This extraordinary expansion in immersion solvency was a consequence of a decrease in molecule size and resulting expansion in surface region. Thus, it very well may be accepted that this expansion in immersion solvency might increment bioavailability.

In-vitro disintegration study
The disintegration profile of optimized formulation of nanosuspension, (Norvir ® Tablet) are introduced in Figure. In nanosuspension, over 97.52 % medication was delivered inside 2 mins, while the 77.94 % at 60 min separately. Thus, nanosuspension upgraded the pace of disintegration of ritonavir by and large.

Evaluation Parameters	Results
Mean Particle Size	287.3 nM
PDI	0.385
Zeta Potential	-26.75 mV
Drug Content	99.98 % w/w
Saturation Solubility	109.8 μg/ml
CPR at 2 mins	97.52 % w/w

Table 12: Evaluation parameter of optimized formulation RF-09

5.14.6 Scanning electron microscopy (SEM)

The surface characteristics of ritonavir and its lyophilized nanosuspension were analysed using a scanning electron microscope (SEM) at magnifications ranging from 3kx to 28kx. The specimens were affixed to carbon adhesive tape on both sides, which had been previously adhered to brass stubs and subsequently coated with gold using a sputter coater for a duration of 4 minutes at a process current of 10 mA. The accelerating voltage was 15 kilovolts. The scanning electron microscopy analysis revealed that the surface morphology of ritonavir is characterised by elongated, slender, and planar structures, with particle dimensions ranging from 4 to 30 nanometers, as depicted in Figure 5.14. Nevertheless, when transformed into lyophilized nanosuspension, the particles that adhered to the mannitol surface as a cryoprotectant became smaller (about 300nm), potentially as a result of hydrophobic interact action.

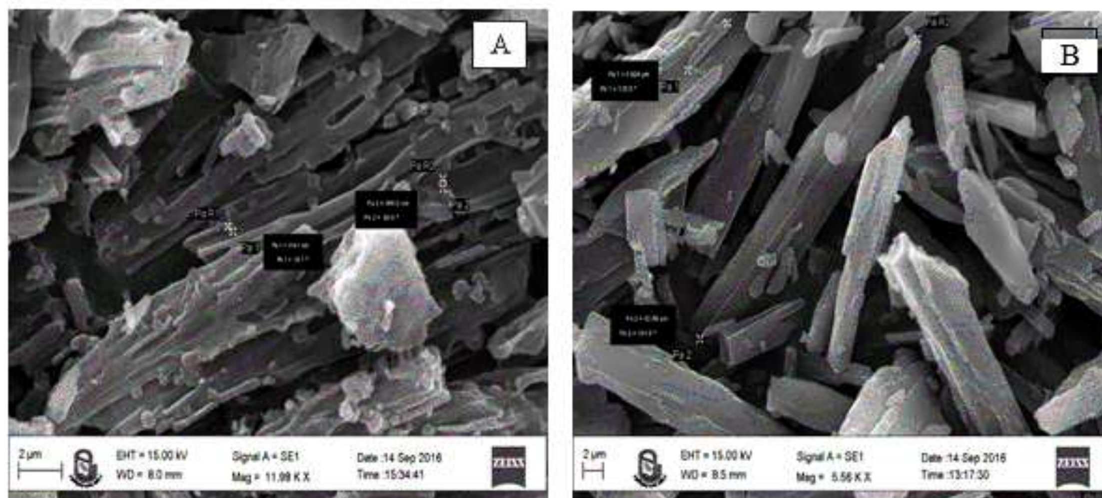

Figure 11: Scanning electron microscopy of (A) Ritonavir and (B) Formulation RF-09

5.14.8. Kinetic order study for optimized formulation RF-09

Time (min)	% cumulative drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released	% Drug released	Cube Root of % drug Remaining (Wt)
0	0	100	0.000	2.000	0.000	0.000	100	4.642
10	4.22	95.78	3.162	1.981	1.000	0.625	4.22	4.575
20	6.52	93.48	4.472	1.971	1.301	0.814	2.3	4.538
30	9.21	90.79	5.477	1.958	1.477	0.964	2.69	4.494

40	13.25	86.75	6.325	1.938	1.602	1.122	4.04	4.427
50	19.12	80.88	7.071	1.908	1.699	1.281	5.87	4.325
60	29.45	70.55	7.746	1.848	1.778	1.469	10.33	4.132
70	46.12	53.88	8.367	1.731	1.845	1.664	16.67	3.777
80	51.32	48.68	8.944	1.687	1.903	1.710	5.2	3.651
90	61.25	38.75	9.487	1.588	1.954	1.787	9.93	3.384
100	72.05	27.95	10.000	1.446	2.000	1.858	10.8	3.035
110	88.12	11.88	10.488	1.075	2.041	1.945	16.07	2.282
120	98.12	1.88	10.954	0.274	2.079	1.992	10	1.234

Table 13 Rrelease kinetics studies of the optimized formulation

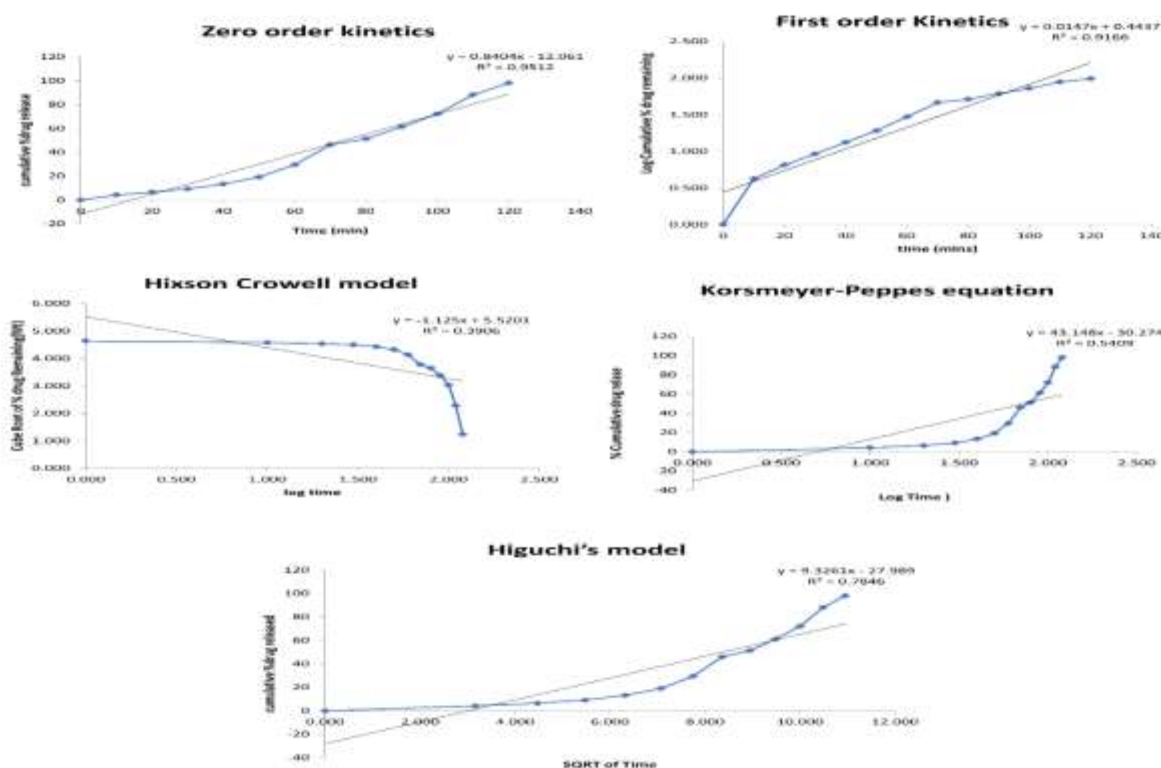

Figure 12Kinetic study graph for the optimized formulation RF-09

Order of kinetics	Zero order	First order	Hixson Crowell model	Korsmeyer -peppas Model	Higuchi plot
Regression value(r2)	0.952	0.916	0.3906	0.549	0.789

The drug release from the optimized formulation for ritonavir nanosuspension was explained employing mathematical equations, including zero-order and first-order approaches. From the regression values, it was determined that the optimized formulation exhibits zero-order drug release. The determined n value of 0.84 indicates that drug release is characterized by non-Fickian transport.

Accelerated stability study of the optimized formulation RF-09

After six months of storage at 25 ±2°C and 60 ± 5% RH, the lyophilized ritonavir nanosuspension was shown to be physically and chemically stable, according to the accelerated stability investigation. The findings for mean particle size, saturation solubility, cumulative percentage release at two minutes, and percentage w/w of drug content are displayed in Table . A little, 5% biassed change was evident in all indicators, but it was not significant. According to the ICH criteria, the optimized batch's results before and after the stability investigation revealed a minor change

S,No	Storage conditions for stability	Time in months	Evaluation parameters			
			Mean particle size (nM) (Mean ±SD)*	Saturation solubility (µg/ml) (Mean ±SD)*	CPR at 2 min (% w/w) (Mean ±SD)*	Drug content (%w/w) (Mean ±SD)*
1	25°C ± 2°C and 60% ± 5% RH	0	286. ±0.23	110.15 ± 2.1	97.90 ± 0.14	102.1± 0.22
2		1	285.6 ± 0.12	111.85 ± 1.2	96.21 ± 1.25	100.5 ± 0.21
3		3	285.2± 0.89	107.82± 2.2	95.92 ± 1.12	99.85 ± 1.24
4		6	282.14 ± 6.5	110.12± 2.2	94.14 ± 1.42	98.52 ± 0.78

Table 14 Accelerated stability study of ritonavir

*Indicates average of three determinations

Summary

The present work is to improve the bioavailability and solubility of poorly soluble drugs with a nanosuspension using a new technology. Nanosuspension is one of those approaches that can greatly improve the effective surface of drug particles and also show a vapor pressure effect and thus increase the dissolution rate

and therefore bioavailability. The main objective of this thesis was to evaluate the possibilities of applying nanosuspension technology in controlled drug release. The main focus was on achieving high and sustained plasma levels *in vivo* by releasing nanosuspension particles from the formulations. This involved preparation, characterization, *in vitro* study and *in vivo* evaluation of several nanosuspension formulations. The aim was to report on unmet needs related to nanosuspension preparation and controlled release formulations of nanoparticles.

Ritonavir is an antiretroviral agent and a BCS class II drug that is poorly soluble in water. Therefore, dissolution is the rate-limiting step to improve the bioavailability of selected drugs. The first part of the study involved the preparation of a nanosuspension of ritonavir using anti-solvent precipitation and then sonication technology to improve oral bioavailability. PVP K-30 was found to be the most suitable stabilizer for ritonavir nanosuspension. The compatibility study was performed using FTIR and DSC studies and showed the compatibility of drugs and excipients. The optimized batch's mean particle size was 287.3 nM, and the PDI was 0.385. Zeta capability of improved detailing was noticed 26.75 ± 3.25 mV which follows the prerequisite of zeta potential. The drug content was by UV-Apparent spectrophotometer at 254 nm and was viewed as 99.98 % w/w of the ritonavir. Immersion dissolvability of a streamlined cluster of ritonavir nanosuspension was found to be 109.8 µg/ml. The disintegration profile of optimized formulation of nanosuspension, (Norvir ® Tablet). In nanosuspension, over 97.52 % medication was delivered inside 2 mins, while the 77.94 % at 60 min separately. The scanning electron microscopy analysis revealed that the surface morphology of ritonavir is characterized by elongated, slender, and planar structures, with particle dimensions ranging from 4 to 30 nanometers. The dissolution data of the optimized formulation was done and studies shows release rate at 120 min shows at 98.2 %.

The Accelerated stability studies shows that Mean particle size (nM) ranging from 241.1 to 282 nM , Saturation solubility (µg/ml) ranging from 107.85 to 111.85, CPR at 2 min (% w/w) ranging from 94.12 to 97.90 and Drug content (%w/w) ranging 98.52 to 102.12 respectively. . From the regression values, it was determined that the optimized formulation exhibits zero-order drug release. The determined n value of 0.84 indicates that drug release is characterized by non-Fickian transport.

4. Conclusion

In this study, Ritonavir nanosuspension was made using the solvent-anti-solvent approach to improve solubility, dissolution, and oral bioavailability. Ritonavir nanosuspension was stabilized with PVP K-30 and Pluronic F68 and dissolved in methanol and water. FTIR & DSC demonstrated no drug-excipient interaction. Formulation RF-09 was optimized for drug content, particle size, and drug release. All formulations had nano-sized particles, according to SEM photographs. Optimized formulation RF-09 has a stable zeta potential of -26.75 mV. Dissolution relies on particle size. Dissolution is faster with smaller particles. The greatest dissolution rate in 2 hours was 98.12% for RF-09. The kinetic studies of the optimized formulation, the regression values exhibit zero-order drug release. The determined n value of 0.84 indicates that drug release is characterized by non-Fickian transport. Based on ICH criteria, the optimized formulation RF-09 showed greater stability at 25°C ± 2°C and 60% ± 5% RH. The solvent-anti-solvent method is a simple and effective way to

generate submicron particles of poorly water-soluble drugs, improving oral bioavailability for commercial production.

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