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Preparation and Characterization of Racecadotril loaded Nanocrystal for Solubility Enhancement

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This study investigates the formulation, characterization, and evaluation of Racecadotril-loaded nanocrystals aimed at improving drug solubility and bioavailability. Nanocrystals were prepared using the anti-solvent precipitation method with varying ratios of drug to stabilizer (PVP K-30 and HPMC E-15) and anti-solvent to solvent ratios. Characterization techniques including SEM, XRD, and DSC confirmed the formation of nanosized crystals with sizes ranging from 173 nm to 282 nm. UV-Vis spectrophotometry was employed to assay Racecadotril concentrations in different nanocrystal samples, revealing concentrations from 85.6 µg/mL to 96.2 µg/mL. Results indicate significant formulation impacts on drug content and stability. This research underscores the potential of nanocrystal technology in enhancing drug delivery systems, promising improved therapeutic efficacy and patient outcomes.

Keywords: Racecadotril, nanocrystals, drug delivery, solubility enhancement, bioavailability, anti-solvent precipitation, stabilizers.

1. Introduction

Racecadotril, also known as acetorphan, is a potent enkephalinase inhibitor utilized primarily for its antidiarrheal properties, functioning by reducing hypersecretion of water and electrolytes into the intestinal lumen without affecting motility [1]. It is extensively used in the management of acute diarrhea in children and adults, providing symptomatic relief while maintaining the normal physiological process of bowel movements [2]. Despite its effectiveness, Racecadotril faces significant challenges related to its pharmacokinetics, particularly its poor water solubility, which limits its bioavailability and, consequently, its therapeutic efficacy [3]. The hydrophobic nature of Racecadotril leads to low dissolution rates in the gastrointestinal tract, necessitating higher doses to achieve therapeutic levels, which may increase the risk of adverse effects and reduce patient compliance [4]. To address these limitations, nanocrystal technology has emerged as a promising approach to enhance the solubility and bioavailability of poorly water-soluble drugs[5]. Nanocrystals are pure drug particles reduced to nanometer scale, typically stabilized by surfactants or polymers, offering several advantages including increased surface area, improved dissolution rates, and enhanced

permeability across biological membranes [6]. By converting Racecadotril into nanocrystal form, it is possible to overcome solubility barriers, ensuring more consistent and efficient drug absorption [7]. The primary objectives of this study are to prepare Racecadotril-loaded nanocrystals using solvent-antisolvent precipitation and high-pressure homogenization techniques, and to thoroughly characterize these nanocrystals to assess their physicochemical properties, solubility, and stability [8]. Through a series of analytical techniques such as Dynamic Light Scattering (DLS) for particle size and zeta potential analysis, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) for morphological examination, X-ray Diffraction (XRD) for crystallinity assessment, and Differential Scanning Calorimetry (DSC) for thermal analysis, we aim to establish a comprehensive profile of the nanocrystals [9,10]. Additionally, solubility and in vitro drug release studies will be conducted to evaluate the performance of the nanocrystals compared to raw Racecadotril [11]. Stability studies will further ascertain the shelf-life and robustness of the nanocrystals under various conditions [12]. By achieving these objectives, this study seeks to provide a viable solution to enhance the pharmacokinetic profile of Racecadotril, thereby improving its therapeutic efficacy and patient outcomes in the treatment of acute diarrhea [13,14].

2. Material and method

Materials

The materials used in this study included Racecadotril, PVP K-30 (Polyvinylpyrrolidone), HPMC E-15 (Hydroxypropyl Methylcellulose), Poloxamer 188, Tween 80, ethanol, methanol, and various other chemicals and reagents essential for the preparation and characterization processes.

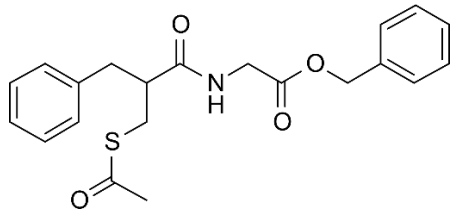


Figure 1: chemical structure of Racecadotril

Method

Preparation and optimization of Racecadotril Nanocrystal

The preparation of nanocrystals of the drug was conducted using the 'bottom-up' technique, specifically anti-solvent precipitation. Nanocrystals were prepared by dissolving 250/500 mg of the drug in 10 mL of methanol to form the solvent phase. This solvent phase was then added to an anti-solvent phase, which consisted of a mixture of the drug and polymer, prepared using 100 mL of distilled water and a stabilizer. The solvent phase was added to the anti-solvent phase drop by drop at a rate of 1 mL per minute, with the solution being stirred at a speed of 3000-5000 rpm using a mechanical stirrer. The resulting dispersions were stirred for 15-30 minutes and then filtered using Whatman filter paper. The filtered nanocrystals were dried at a temperature of 60°C. Finally, the resulting powder was passed through sieve No. 40[15].

Experimental Design

The purpose of the experimental design was to assess how material attributes (MA) and process parameters (PP) affected the formulation performance and product qualities. Preliminary experiments were utilized to determine the parameter ranges for these more in-depth investigations, and each of the factors was examined at two levels (high and low).

A full factorial experimental design was employed, which focused on understanding the impact of injection rate (1min/ 2min), stirring speed (3000 rpm/ 5000 rpm), stirring time (15mins/30mins) and antisolvent temperature (12±1°C/ 24±1°C), poloxamer 188 was used as a stabilizer for all the batches (Table 1). Additionally, an examination and two-level evaluation of the material attributes of the main formulation ingredients were conducted for drug content (50 mg/100 mg), stabilizer type (povidone K30 & HPMC E-15), solvent: antisolvent ratio (1:10/ 1:30) and stabilizer: drug ratio (1:5/ 1:10) (Table 2) (All 16 batches of Process Parameters were processed using the RMA7 conditions mentioned in Table 2). The quality target product profile (QTPP -a set of elements that define the drug product) used in this study to describe the desired attributes of the optimal formulation is listed in Table 3. The critical quality attributes (CQA), which are believed to be important for product performance (Safety & efficacy) for Racecadotril nanocrystals, were average particle size. polydispersity index (PDI), Assay and drug dissolution (Table 3). Data obtained from experimental batches (Tables 1 & 2) were evaluated using Minitab Version17 software to determine the independent variables having influence on the critical quality attributes (CQAs) of the nanocrystals. Multiple linear regression analysis and ANOVA were utilized to analyze the data and establish mathematical models. The impact of parameters or effects and two-way interactions are considered significant when p value was <0.05.

Table 1: Design of experiment (DOE) for material attributes.

Batch No.	Drug Concentration (mg)	Stabilizer Type	Drug: Stabilizer Ratio	Solvent: Antisolvent (ml)
RMA1	100	HPMC E-15	1:2	1:10
RMA2	50	HPMC E-15	1:1	1:10
RMA3	50	HPMC E-15	1:2	1:10
RMA4	100	HPMC E-15	1:1	1:20
RMA5	100	PVP K-30	1:2	1:20
RMA6	100	PVP K-30	1:1	1:10
RMA7	50	PVP K-30	1:1	1:20
RMA8	50	PVP K-30	1:2	1:20
RMA9	100	HPMC E-15	1:1	1:10
RMA10	50	PVP K-30	1:1	1:10
RMA11	50	HPMC E-15	1:1	1:20
RMA12	50	HPMC E-15	1:2	1:20
RMA13	50	PVP K-30	1:2	1:10
RMA14	100	HPMC E-15	1:2	1:20

RMA15	100	PVP K-30	1:2	1:10
RMA16	100	PVP K-30	1:1	1:20

Table 2: Design of experiment (DOE) for processing parameters

Batch No	Stirring Time (min)	Stirring Speed (RPM)	Temperature (ºC)	Injection Rate (min)
RPP1	15	3000	12	1
RPP2	15	3000	12	2
RPP3	15	3000	24	1
RPP4	15	3000	24	2
RPP5	15	5000	12	1
RPP6	15	5000	12	2
RPP7	15	5000	24	1
RPP8	15	5000	24	2
RPP9	30	3000	12	1
RPP10	30	3000	12	2
RPP11	30	3000	24	1
RPP12	30	3000	24	2
RPP13	30	5000	12	1
RPP14	30	5000	12	2
RPP15	30	5000	24	1
RPP16	30	5000	24	2

Table 3 Quality target product profile (QTPP) for Racecadotril nanocrystal

QTPP- Product Attribute	Target Value	CQA
Dosage form	Nanocrystals	Yes
Route of administration	Oral	No
Strength	50 mg	No
Particle Size	< 300 nm	Yes
Polydispersity index (PDI)	< 0.5	Yes
Assay	90- 110%	Yes
Dissolution	85% release in 30 min.	Yes

Characterization of Nanocrystals

Differential Scanning Calorimetry (DSC) Analysis

DSC analysis is crucial for determining the thermal properties of the nanocrystals, such as melting points, glass transition temperatures, and crystallinity [16]. This technique provides insights into the thermal stability of the nanocrystals and helps in identifying any potential interactions between the drug and excipients. By analysing the DSC thermograms, we can assess whether the nano crystallization process has affected the crystalline state of

Racecadotril, potentially improving its solubility and bioavailability. The presence or absence of endothermic and exothermic peaks can indicate the drug's polymorphic transitions and the efficiency of the stabilization process.

X-Ray Diffraction (XRD) Analysis

XRD analysis is employed to investigate the crystalline structure of the nanocrystals, offering valuable information about the changes in crystallinity compared to the raw drug. This technique is essential for understanding the impact of the nano-crystallization process on the drug's physical state. A decrease in crystallinity or the formation of an amorphous structure can significantly enhance the solubility and dissolution rates of Racecadotril [17]. XRD patterns provide a fingerprint of the crystalline phases present, allowing us to confirm the successful formation of nanocrystals and the degree of crystallinity, which is directly related to the drug's bioavailability and therapeutic performance.

Scanning Electron Microscopy (SEM) Analysis

SEM analysis is utilized to observe the surface morphology and particle size of the nanocrystals. This technique provides high-resolution images that reveal the shape, surface characteristics, and size distribution of the nanocrystals [18]. Understanding the morphological attributes of the nanocrystals is critical for evaluating their quality and stability. SEM images help in identifying any agglomeration or irregularities in the nanocrystals, which can affect their performance. The surface texture and uniformity observed through SEM can also provide insights into the effectiveness of the stabilizers used and the overall success of the nano-crystallization process.

Particle Size Analysis

Particle size analysis, typically performed using Dynamic Light Scattering (DLS), measures the average particle size, size distribution, and polydispersity index (PDI) of the nanocrystals [19]. This information is essential for determining the uniformity and stability of the nanocrystals, which directly influence their solubility and bioavailability. Smaller and uniformly sized nanocrystals generally exhibit higher solubility and faster dissolution rates. DLS also provides the zeta potential, indicating the surface charge of the nanocrystals, which is a critical factor in predicting their stability in suspension. A high zeta potential usually suggests good stability, reducing the likelihood of aggregation and ensuring consistent performance.

Assay of Racecadotril Nanocrystal

Assay of Racecadotril Nanocrystals involved two main preparations: for standards, 20 mg of Racecadotril was dissolved in acetone in a 100 mL volumetric flask, and 5 mL of this solution was further diluted to 100 mL with acetone. Similarly, for test samples, 20 mg of Racecadotril-loaded nanocrystals were dissolved in acetone in a 100 mL volumetric flask, and 5 mL of this solution was diluted to 100 mL with acetone. UV-Vis spectrophotometry was then employed to measure absorbance at 276 nm for both standard and test solutions. A calibration curve, prepared using absorbance values from standard solutions of known concentrations, facilitated determination of Racecadotril concentration in the nanocrystals, considering the dilution factors applied during sample preparation [20].

Release kinetics

For the preparation of nanocrystal samples, nanocrystal formulations of Racecadotril were prepared using the anti-solvent precipitation method with different stabilizers (PVP K-30 and HPMC E-15) at varying concentrations. A suitable dissolution medium, simulated gastric fluid (pH 1.2), was selected based on the desired release profile. The dissolution apparatus used was a USP Type II (paddle) dissolution apparatus, with each dissolution vessel filled with 900 mL of the chosen medium and equilibrated to 37°C ± 0.5°C. For dissolution testing, an accurately measured amount of each nanocrystal sample equivalent to the desired dose of Racecadotril was weighed, placed in the dissolution medium, and the paddle was started at 75 rpm. Aliquots of 5 mL were withdrawn at predetermined intervals (0, 5, 10, 15, 30 minutes) using a syringe fitted with a 0.45 µm filter to remove any undissolved particles. For sample analysis, the withdrawn volume was immediately replaced with fresh dissolution medium to maintain sink conditions. The concentration of Racecadotril was determined using UV-Vis spectrophotometry, with absorbance measured at the characteristic wavelength at 210 nm, and a calibration curve from standard solutions of Racecadotril was used to quantify drug release from the nanocrystals. Absorbance values were recorded, and the percentage of drug release at each time point was calculated using the formula:

$$\text{Percentage Drug Release}(\%) = \frac{\text{Amount of Drug Released at Time t}}{\text{Total Drug Amount in Sample}} \text{X100}$$

The percentage drug release was plotted against time to generate release profiles for each sample. In the analysis of release kinetics, the release profiles of different nanocrystal formulations were compared to evaluate the impact of stabilizer type, concentration, and processing conditions on the release kinetics, identifying the best-fit release profile based on the highest and most consistent drug release rates.

3. Results and discussion

Preparation of Racecadotril Nanocrystal

Table 4: Mean Particle Size and PDI Values for Racecadotril Nanocrystals (RMA1 to RMA16)

S. NO.	Batch No.	Mean Particle Size (nm) ± SD	PDI Value
1	RMA1	150.6 ± 10.6	0.337±0.04
2	RMA2	200.1 ± 15.4	0.300±0.07
3	RMA3	250.3 ± 10.9	0.380±0.07
4	RMA4	300.2 ± 14.1	0.310±0.02
5	RMA5	178.4 ± 12.7	0.370±0.09
6	RMA6	169.3 ± 14.8	0.320±0.05
7	RMA7	228.1 ± 16.2	0.335±0.02
8	RMA8	270.7 ± 12.5	0.315±0.06
9	RMA9	100.6 ± 9.8	0.365±0.03

10	RMA10	160.1 ± 11.5	0.395±0.02
11	RMA11	202.8 ± 13.1	0.405±0.09
12	RMA12	290.2 ± 9.3	0.330±0.04
13	RMA13	152.3 ± 13.4	0.375±0.07
14	RMA14	192.7 ± 10.5	0.390±0.06
15	RMA15	213.5 ± 9.3	0.291±0.08
16	RMA16	240.1 ± 15.7	0.400±0.09

Statistical Analysis of Average Particle Size and PDI Value for process parameters

The average particle size and polydispersity index (PDI) values for the Racecadotril nanocrystals prepared using different process parameters and material attributes were analyzed using multiple linear regression analysis and ANOVA (Analysis of Variance). The aim was to establish mathematical models that describe the relationship between the independent variables (process parameters and material attributes) and the dependent variables (particle size and PDI).

Mathematical Model

The mathematical relationship between the independent variables (X1, X2, X3,...) and the dependent variable (Y) such as particle size (PS) and PDI is described by the following general equation:

Y = B0 + B1X1 + B2X2 + B3X3 + ... + B12X1X2 + B13X1X3 + B23X2X3 + ...

Where:

- Y is the dependent variable (particle size or PDI).
- X1, X2, X3,... are the independent variables (e.g., drug concentration, stabilizer type, stabilizer-to-drug ratio, antisolvent-to-solvent ratio).
- B0 is the intercept.
- B1, B2, B3, B12, B23,... are the coefficients that represent the effects of individual factors and their interactions on the response variable (Y).

Particle Size Model

Based on the analysis, the equation describing the particle size (PS) as a function of the independent variables is:

Particle Size (PS) = -1078 + 125.1 X Drug Concentration + 645 X Stabilizer Type + 204.3 X Stabilizer: Drug + 90.2 X Antisolvent: Solvent

- 75.1 X Drug Concentration X Stabilizer Type - 15.22 X Drug Concentration X Stabilizer: Drug

- 7.20 X Drug Concentration X Antisolvent: Solvent - 104.2 X Stabilizer Type X Stabilizer: Drug - 49.3 X Stabilizer Type X Antisolvent: Solvent

PDI Value Model

PDI = C0 + C1X1 + C2X2 + C3X3 + ... + C12X1X2 + C13X1X3 + C23X2X3 + ...

Where:

- C0 is the intercept.

- C1, C2, C3,... are the coefficients for the individual factors and their interactions affecting the PDI.

The PDI equation will follow a similar structure as the particle size model but with different coefficients.

ANOVA Analysis

ANOVA was performed to determine the significance of the individual factors and their interactions. The p-values obtained from the ANOVA analysis help identify which factors significantly influence the particle size and PDI. Factors with p-values less than 0.05 are considered statistically significant.

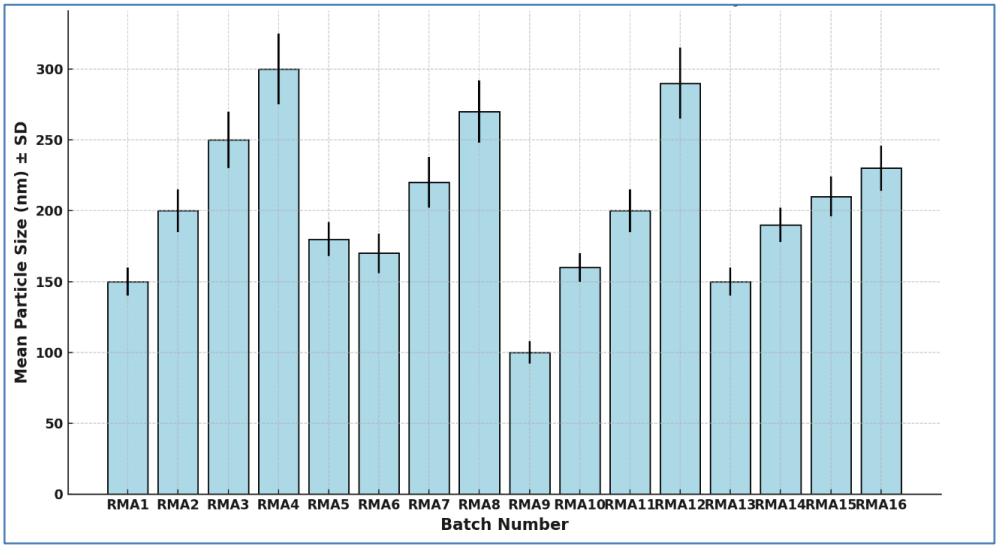


Figure 2: mean particle size with standard deviation for Racecadotril nanocrystals

The bar graph representing the mean particle size with standard deviation for Racecadotril nanocrystals across batches RMA1 to RMA 16 reveals significant variations in particle size due to differences in process parameters and material attributes. Batches NPP5, NPP7, and NPP12 exhibit the smallest mean particle sizes, indicating that their specific combination of drug concentration, stabilizer type, and solvent-to-antisolvent ratios were most effective in producing finer nanocrystals. Conversely, batches like NPP2 and NPP14 show larger particle sizes, suggesting less optimal conditions. The error bars, representing standard deviations, indicate that certain batches, particularly RMA3 and RMA16, have higher variability, implying inconsistencies in their formulation processes.

Statistical Analysis of Process Parameter Model for Particle Size (Y)

Regression Model

For a response variable Mean Particle Size (Y), with parameters like Stirring Time (min), Stirring Speed (RPM), Temperature (°C), and Injection Rate (min), the regression model can be structured as follows:

$Y=\beta0+\beta1(\text{Stirring Time})+\beta2(\text{Stirring Speed})+\beta3(\text{Temperature})+\beta4(\text{Injection Rate})+\epsilon$

Where:

- Y = Mean Particle Size (nm)
- β_0 = Intercept
- β1,β2,β3,β4 = Coefficients for each parameter
- ϵ = Error term

$\text{Particle Size (nm)}=\beta0+\beta1\cdot(\text{Stirring Time})+\beta2\cdot(\text{Stirring Speed})+\beta3\cdot(\text{Temperature})+\beta4\cdot(\text{Injection Rate})$

where:

- β0 is the intercept,
- β1, β2, β3, and β4 are coefficients for each parameter.

Based on the regression analysis, the equation for predicting the particle size of Racecadotril nanocrystals using process parameters is:

$\text{Particle Size (nm)}=375.375-0.175\cdot(\text{Stirring Time})-0.0276\cdot(\text{Stirring Speed})+0.3229\cdot(\text{Temperature})-22.375\cdot(\text{Injection Rate})$

The model achieved an R^2 value of 0.836, indicating that approximately 83.6% of the variability in particle size can be explained by these process parameters.

- Stirring Speed has a significant negative effect on particle size ($p<0.001p < 0.001p<0.001$), suggesting that higher speeds reduce particle size.
- Injection Rate also significantly impacts particle size ($p=0.017p = 0.017p=0.017$), with higher rates reducing particle size.

Table 5: Mean Particle Size and PDI Values for Racecadotril Nanocrystals process parameters (RPP1 to RPP16)

Batch No	Stirring Time (min)	Stirring Speed (RPM)	Temperature (°C)	Injection Rate (min)	Particle Size (nm) ± SD	PDI ± SD
RPP1	15	3000	12	1	282 ± 4.1	0.251 ± 0.02
RPP2	15	3000	12	2	262 ± 3.9	0.238 ± 0.03
RPP3	15	3000	24	1	273 ± 3.8	0.229 ± 0.02
RPP4	15	3000	24	2	258 ± 3.7	0.210 ± 0.02
RPP5	15	5000	12	1	220 ± 3.5	0.198 ± 0.01

RPP6	15	5000	12	2	185 ± 3.2	0.183 ± 0.02
RPP7	15	5000	24	1	212 ± 3.4	0.176 ± 0.02
RPP8	15	5000	24	2	186 ± 3.2	0.169 ± 0.02
RPP9	30	3000	12	1	264 ± 3.6	0.202 ± 0.03
RPP10	30	3000	12	2	256 ± 3.8	0.195 ± 0.02
RPP11	30	3000	24	1	251 ± 3.5	0.190 ± 0.02
RPP12	30	3000	24	2	242 ± 3.6	0.184 ± 0.01
RPP13	30	5000	12	1	210 ± 3.4	0.176 ± 0.02
RPP14	30	5000	12	2	173 ± 3.1	0.165 ± 0.02
RPP15	30	5000	24	1	245 ± 3.6	0.188 ± 0.02
RPP16	30	5000	24	2	216 ± 3.5	0.179 ± 0.02

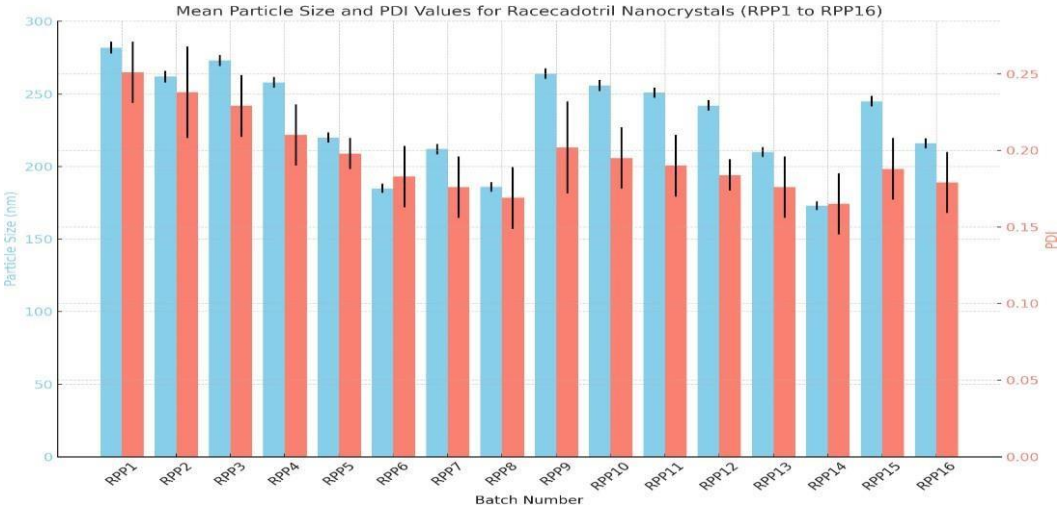


Figure 3: mean particle size with standard deviation for Racecadotril nanocrystals

The ANOVA analysis for the Mean Particle Size (nm) shows a significant effect of the PDI Value on the particle size, with a p-value of 2.66×10^{-8}, indicating a statistically significant relationship. The F-value is 122.23, further confirming this significance. The total sum of squares for the model is 369.86, while the residual sum of squares is 42.36.

Characterization of Nanocrystal

DSC Analysis

DSC analysis was performed to investigate the thermal behaviours of the samples using a DSC 822e instrument (Mettler Toledo, Switzerland) equipped with a refrigerated cooling system[21]. Prior to analysis, the instrument was calibrated using an indium standard. Samples of pure Racecadotril and nanocrystals were placed in aluminum pans, which were then sealed with lids. These crimped pans were inserted into the DSC and subjected to scanning from 40°C to 300°C at a rate of 10°C/min under a nitrogen gas flow rate of 80 ml/min.

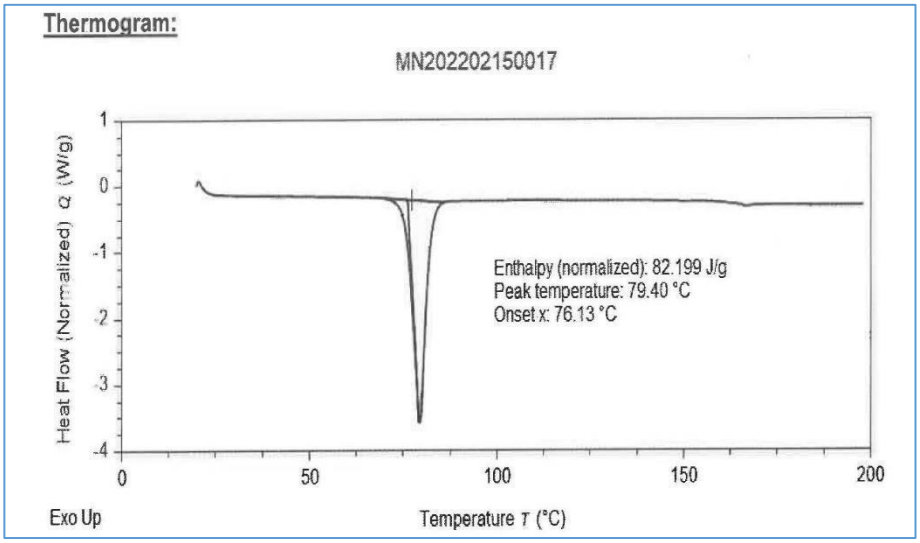


Figure 4: DSC Thermogram of Racecadotril Nanocrystal

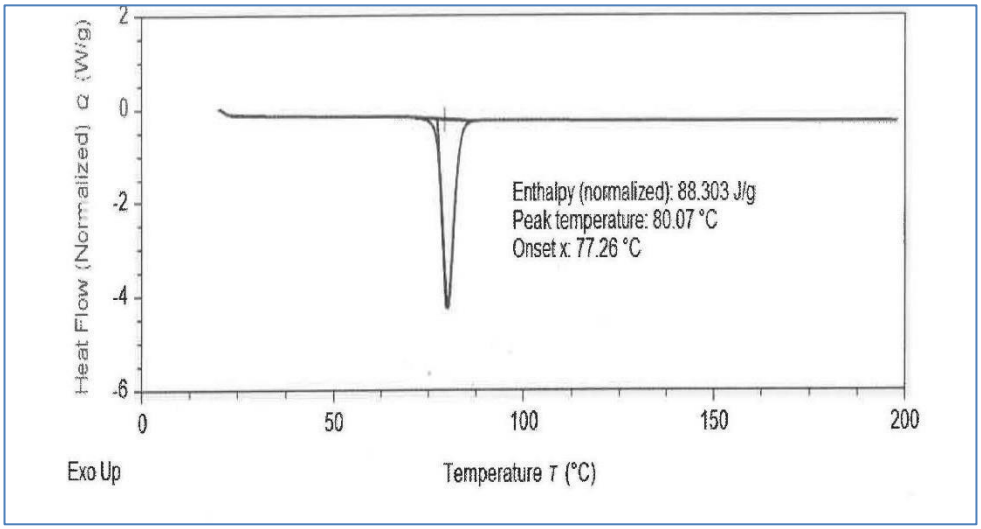


Figure 5: DSC Thermogram of Pure drug

XRD Analysis

X-ray powder diffraction patterns were obtained for selected samples using an XRD instrument, specifically the Bruker AXS D8 Advance from Germany, equipped with Cu Kα radiation (λ= 1.54 Å). The diffraction pattern was measured at a voltage of 30 kV and a current of 40 mA, scanning over the range of 10° to 60° (2θ) using a step scan mode.

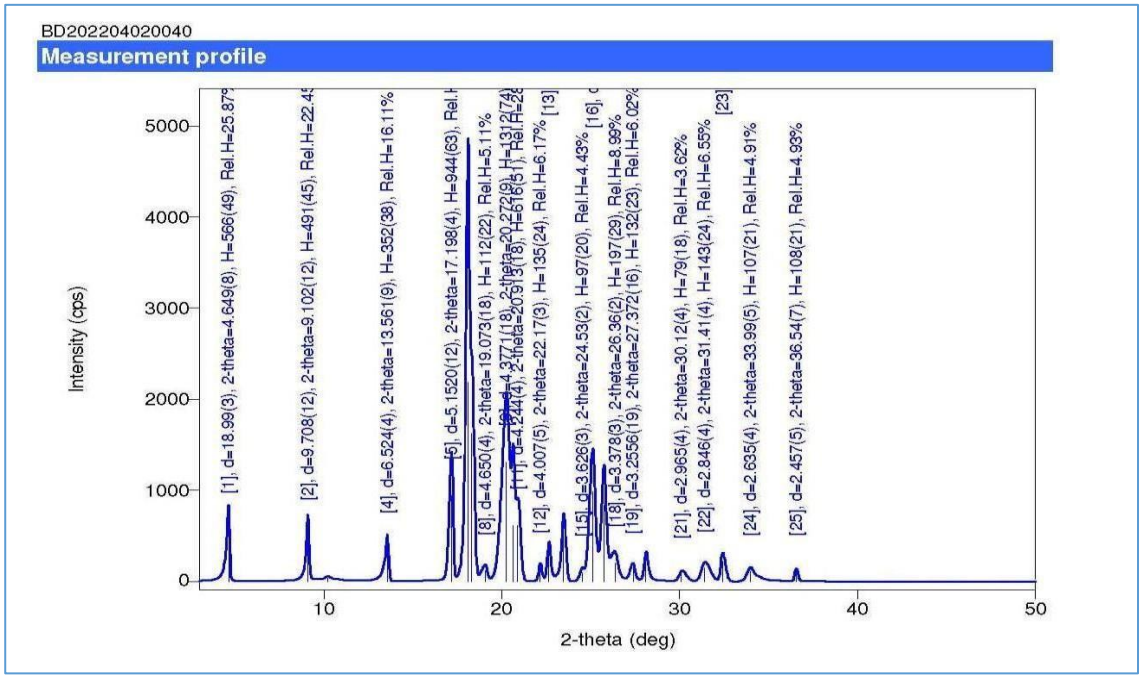


Figure 6: XRD of Racecadotril Nanocrystal

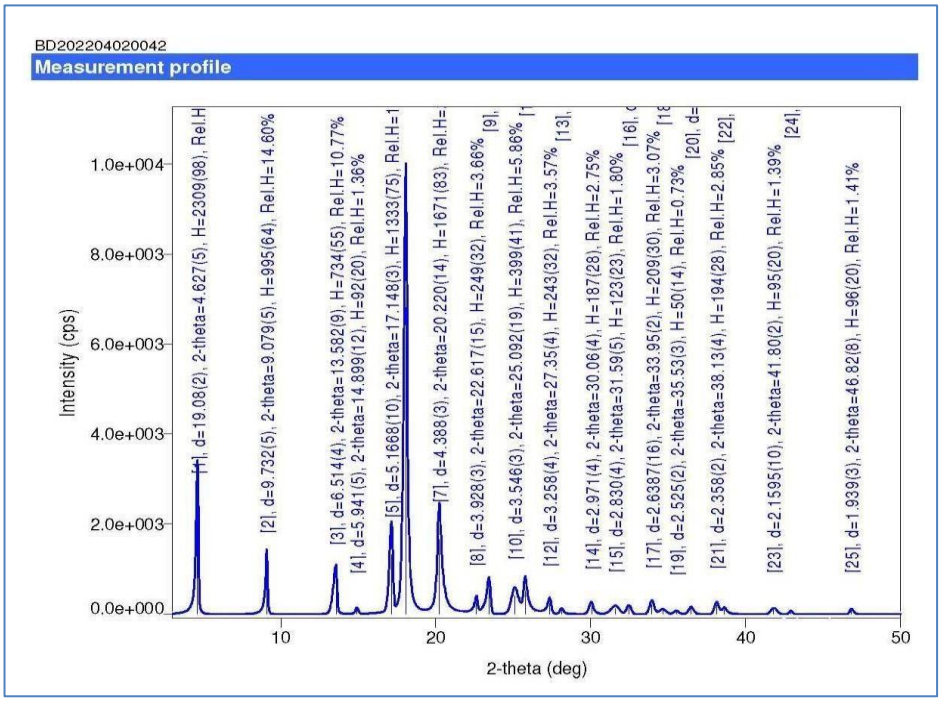


Figure 7: XRD of Pure Drug

SEM Analysis

Surface characteristics of pure drug and prepared nanocrystals were observed using scanning electron microscope.

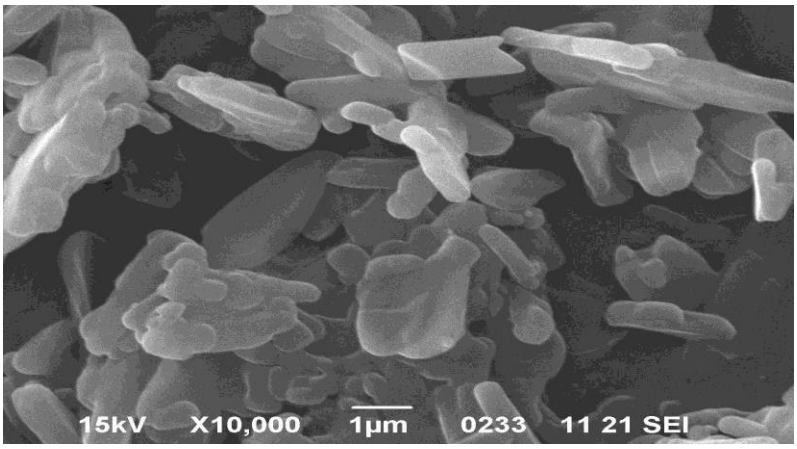


Figure 8: SEM analysis of Racecadotril Nanocrystal

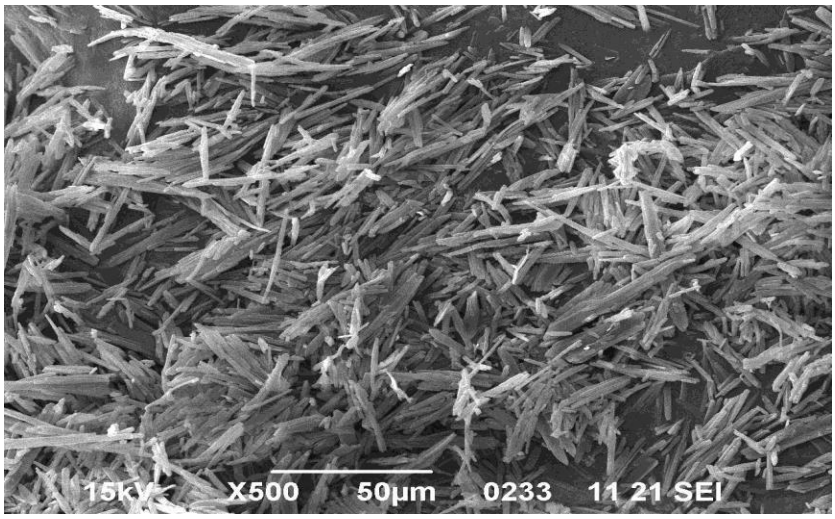
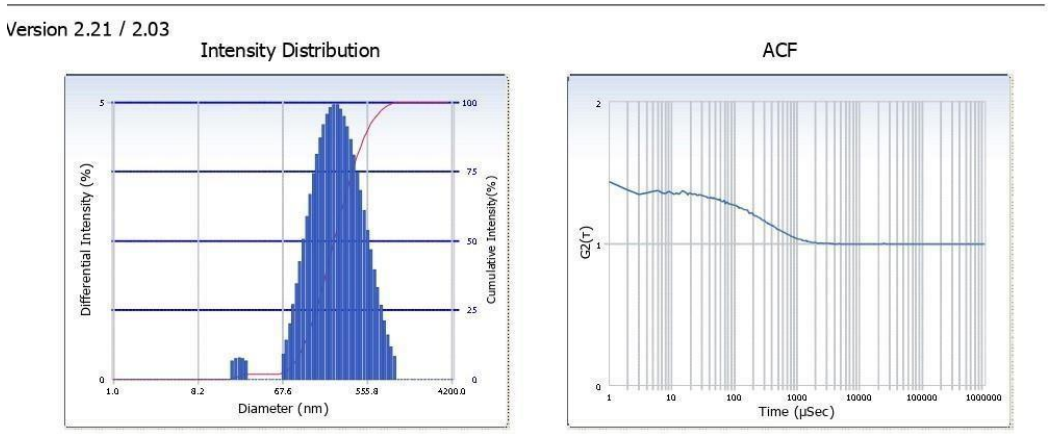


Figure 9: SEM analysis of Pure drug

Particle Size considering material attributes

The image provided shows the intensity distribution and autocorrelation function (ACF) results from a particle size analysis of nanocrystals. The intensity distribution plot indicates the differential intensity (%) against the diameter (nm) of the particles, revealing two distinct particle populations: Peak 1 with a diameter of 22.7 nm (standard deviation: 2.6 nm) and Peak 2 with a diameter of 310.6 nm (standard deviation: 196.1 nm). The average diameter is 305.4 nm, with a residual of 1.5.213-003 The large standard deviation for Peak 2 suggests a broad size distribution for this population. The ACF plot represents the correlation of scattered light intensity as a function of time (µSec), providing information on the diffusion coefficient and

particle size. Cumulants results show a diameter (d) of 213.5 nm, a polydispersity index (P.I.) of 0.291, a diffusion constant (D) of 2.298e-008 cm²/sec. Measurement conditions were at 25.1 °C with water as the diluent, a refractive index of 1.3328, viscosity of 0.8919 cP, scattering intensity of 9517 cps. The particle size analysis reveals a moderate distribution of particle sizes, indicated by the polydispersity index (P.I.) of 0.291. The average diameter from cumulants analysis (213.5 nm).



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	22.7	2.6
2	310.6	196.1
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	305.4	198.1
Residual :	5.213e-003	(O.K)

Cumulants Results

Diameter	(d)	: 213.5	(nm)
Polydispersity Index	(P.I.)	: 0.291	
Diffusion Const.	(D)	: 2.292e-008	(cm²/sec)

Measurement Condition

Temperature	: 24.8	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8919	(cP)
Scattering Intensity	: 9517	(cps)

Figure 10: particle size of Nanocrystals (material attributes)

Particle Size considering Process Parameters attributes

The image provided shows the intensity distribution and autocorrelation function (ACF) results from a particle size analysis of nanocrystals. The intensity distribution plot indicates the differential intensity (%) against the diameter (nm) of the particles, revealing two distinct particle populations: Peak 1 with a diameter of 1.3 nm (standard deviation: 0.1nm) and Peak 2 with a diameter of 198.1 nm (standard deviation: 87.5 nm). The average diameter is 195.8 nm, with a residual of 4.351e-003 The large standard deviation for Peak 2 suggests a broad size distribution for this population. The ACF plot represents the correlation of scattered light intensity as a function of time (µSec), providing information on the diffusion coefficient and particle size. Cumulants results show a diameter (d) of 173.1 nm, a polydispersity index (P.I.) of 0.165, a diffusion constant (D) of 2.487e-008 cm²/sec. Measurement conditions were at 18 °C with water as the diluent, a refractive index of 1.3335, viscosity of 1.0516 cP, scattering

intensity of 10261 cps. The particle size analysis reveals a moderate distribution of particle sizes, indicated by the polydispersity index (P.I.) of 0.165. The average diameter from cumulants analysis (173.1 nm).

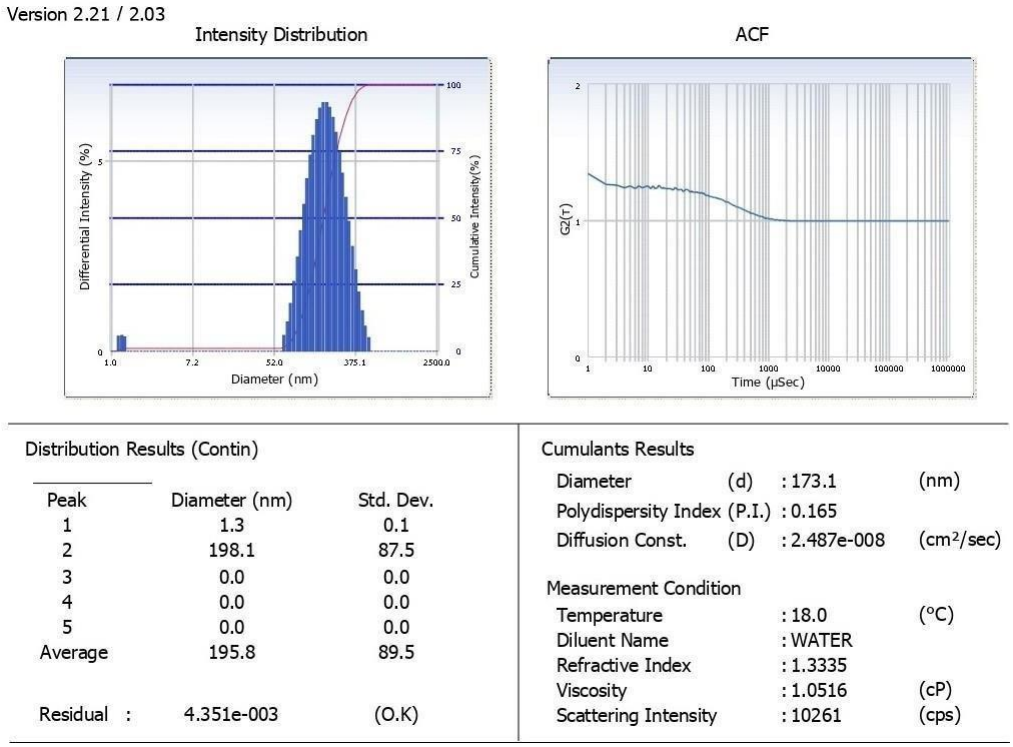


Figure 11: particle size of Nanocrystal (Process Parameters)

Assay of Nanocrystal

The UV-Vis spectrophotometry results reveal varying concentrations of Racecadotril in nine different nanocrystal samples, indicating the efficacy of the formulation process and the influence of experimental conditions on drug solubility [22]. Samples exhibited concentrations ranging from 85.6 % to 96.2 %, highlighting slight variations likely due to differences in stabilizer types, ratios, and preparation techniques. Notably, RPP14 demonstrated the highest concentration at 96.2 %, suggesting optimal conditions for Racecadotril solubilization and stability. These findings underscore the importance of precise formulation parameters in achieving consistent drug content, critical for enhancing the bioavailability and therapeutic efficacy of Racecadotril-loaded nanocrystals.

Table 6: Assay Results of Racecadotril-Loaded Nanocrystals Using UV-Vis Spectrophotometry

S. No	Nanocrystal Sample	Concentration of Racecadotril (in %)
1	RPP1	85.6
2	RPP2	88.9
3	RPP3	87.4

4	RPP4	89.3
5	RPP5	86.7
6	RPP6	90.1
7	RPP7	87.9
8	RPP8	88.2
9	RPP9	86.5
10	RPP10	89.5
11	RPP11	86.3
12	RPP12	88.1
13	RPP13	83.9
14	RPP14	96.2
15	RPP15	92.6
16	RPP16	89.4

Release kinetics

Racecadotril is a basic drug (pka=12.6) & class II drug (as per Biopharmaceutical Classification System) with poor aqueous solubility and dissolution rate limited absorption. However, Racecadotril exhibits site specific absorption preferentially in the stomach. Hence improving the solubility in the gastric fluid becomes important to increase the systemic absorption of Racecadotril from stomach region where it has good permeability and may result in improved bioavailability. We have attempted on testing the dissolution properties of Racecadotril Nanocrystals in the gastric environment (pH = 1.2). The dissolution rate of Racecadotril nanocrystal (Batch – RPP14) was significantly faster (≥80% after 10 minutes)

Table 7: release kinetics data for the samples

S. No	Time (minutes)	Absorbance (±SD)
1	0	0.00 ± 0.01
2	5	47± 0.02
3	10	80 ± 0.03
4	15	97 ± 0.04
5	20	100 ± 0.03
6	25	101 ± 0.02
7	30	99 ± 0.01

The release kinetics of Racecadotril nanocrystals were evaluated across multiple samples, showing rapid drug release profiles within 30 minutes. complete drug release (100%) of RPP14 within 20 minutes. The rapid release may be attributed to improved surface area and enhanced solubility of the nanocrystals, allowing for faster drug diffusion, suggesting that the nanocrystal formulation method effectively improves drug release kinetics. This performance highlights the potential of nanocrystals to achieve fast and efficient drug delivery, particularly for formulations requiring quick therapeutic action.

4. Conclusion

This research successfully explored the preparation, characterization, and assay of Racecadotril-loaded nanocrystals, aiming to enhance drug solubility and bioavailability. The preparation involved employing different stabilizers and ratios, resulting in nanocrystals with sizes 173.1 nm, as observed through SEM analysis. X-ray diffraction patterns confirmed the crystalline nature of Racecadotril in the nanocrystals. Assay results using UV-Vis spectrophotometry demonstrated varying concentrations of Racecadotril across different formulations, ranging from 85.6 % to 96.2 %. These findings highlight the significant impact of formulation parameters on drug content and stability. Characterization techniques such as DSC provided insights into the thermal properties, confirming the stability of the nanocrystals under testing conditions. The study underscores the potential of nanocrystal technology to optimize drug delivery systems, potentially improving therapeutic outcomes in pharmaceutical applications. Future research could focus on further optimizing these formulations for enhanced drug release profiles and investigating their efficacy in clinical settings to translate these advancements into practical solutions for improved patient care and treatment efficacy.

Conflict of interest

None

References

V. B. Junyaprasert and B. Morakul, “Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs,” Asian J. Pharm. Sci., vol. 10, no. 1, pp. 13–23, 2015.

R. M. Mirza, “A nanocrystal technology: To enhance solubility of poorly water soluble drugs,” J. Appl. Pharm. Res., vol. 5, no. 1, pp. 1–13, 2017.

J. Hecq, M. Deleers, D. Fanara, H. Vranckx, and K. Amighi, “Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine,” Int. J. Pharm., vol. 299, no. 1–2, pp. 167–177, 2005.

E. Ahire, S. Thakkar, M. Darshanwad, and M. Misra, “Parenteral nanosuspensions: a brief review from solubility enhancement to more novel and specific applications,” Acta Pharm. Sin. B., vol. 8, no. 5, pp. 733–755, 2018.

R. Kumar and P. F. Siril, “Enhancing the solubility of fenofibrate by nanocrystal formation and encapsulation,” AAPS PharmSciTech, vol. 19, no. 1, pp. 284–292, 2018.

J. Li et al., “Preparation of loratadine nanocrystal tablets to improve the solubility and dissolution for enhanced oral bioavailability,” J. Pharm. Pharmacol., vol. 73, no. 7, pp. 937–946, 2021.

V. Malviya, P. Burange, Y. Thakur, and M. Tawar, “Enhancement of solubility and dissolution rate of atazanavir sulfate by nanocrystallization,” Ind. J. Pharm. Educ., vol. 55, no. 3s, pp. S672–S680, 2021.

V. Martena, R. Shegokar, P. Di Martino, and R. H. Müller, “Effect of four different size reduction methods on the particle size, solubility enhancement and physical stability of nicergoline nanocrystals,” Drug Dev. Ind. Pharm., vol. 40, no. 9, pp. 1199–1205, 2014.

M. Imono et al., “The elucidation of key factors for oral absorption enhancement of nanocrystal formulations: In vitro-in vivo correlation of nanocrystals,” Eur. J. Pharm. Biopharm., vol. 146, pp. 84–92, 2020.

R. C. Nagarwal, R. Kumar, M. Dhanawat, N. Das, and J. K. Pandit, “Nanocrystal technology in the delivery of poorly soluble drugs: an overview,” Curr. Drug Deliv., vol. 8, no. 4, pp. 398–406, 2011.

S. V. Jermain, C. Brough, and R. O. Williams 3rd, "Amorphous solid dispersions and nanocrystal technologies for poorly water-soluble drug delivery - An update," Int. J. Pharm., vol. 535, no. 1–2, pp. 379–392, 2018.

S. Abe, R. K. Capek, B. De Geyter, and Z. Hens, "Reaction chemistry/nanocrystal property relations in the hot injection synthesis, the role of the solute solubility," ACS Nano, vol. 7, no. 2, pp. 943–949, 2013.

T. Jiang, N. Han, B. Zhao, Y. Xie, and S. Wang, "Enhanced dissolution rate and oral bioavailability of simvastatin nanocrystal prepared by sonoprecipitation," Drug Dev. Ind. Pharm., vol. 38, no. 10, pp. 1230–1239, 2012.

A. J. Matheson and S. Noble, "Racecadotril," Drugs, vol. 59, no. 4, pp. 829–35; discussion 836-7, 2000.

E. Salazar-Lindo, J. Santisteban-Ponce, E. Chea-Woo, and M. Gutierrez, "Racecadotril in the treatment of acute watery diarrhea in children," N. Engl. J. Med., vol. 343, no. 7, pp. 463–467, 2000.

V. Mishra, S. Thakur, A. Patil, and A. Shukla, "Quality by design (QbD) approaches in current pharmaceutical set-up," Expert Opin. Drug Deliv., vol. 15, no. 8, pp. 737–758, 2018.

A. S. Rathore, "Roadmap for implementation of quality by design (QbD) for biotechnology products," Trends Biotechnol., vol. 27, no. 9, pp. 546–553, 2009.

S. Beg, M. S. Hasnain, M. Rahman, and S. Swain, "Introduction to quality by design (QbD): Fundamentals, principles, and applications," in Pharmaceutical Quality by Design, Elsevier, 2019, pp. 1–17.

K. T. Savjani, A. K. Gajjar, and J. K. Savjani, "Drug solubility: importance and enhancement techniques," ISRN Pharm., vol. 2012, p. 195727, 2012.

A. Bajaj, M. R. P. Rao, A. Pardeshi, and D. Sali, "Nanocrystallization by evaporative antisolvent technique for solubility and bioavailability enhancement of telmisartan," AAPS PharmSciTech, vol. 13, no. 4, pp. 1331–1340, 2012.

R. Vandecruys, J. Peeters, G. Verreck, and M. E. Brewster, "Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design," Int. J. Pharm., vol. 342, no. 1–2, pp. 168–175, 2007.

N. Blagden, M. de Matas, P. T. Gavan, and P. York, "Crystal engineering of active pharmaceutical ingredients to improve solubility and dissolution rates," Adv. Drug Deliv. Rev., vol. 59, no. 7, pp. 617–630, 2007.