Section A-Research paper

Development of electro-sterically stabilized alvimopan nanocrystals to improve oral bioavailability by wet media milling technique: formulation optimization using factorial design, characterization, in *vitro* - in *vivo* evaluation

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Abstract:

Alvimopan (ALV), a medication for the treatment of postoperative ileus, is a biopharmaceutical classification system (BCS) class IV drug whose poor aqueous solubility restricts its bioavailability. The aim of the research was to create nanocrystals that would help ALV dissolve more quickly and be more bioavailable when taken orally. To improve oral bioavailability, the ALV nanocrystals (ALV-NC) were made using a wet media milling technique and fluidized bed processing. In, formulation screening, sodium lauryl sulphate (SLS) was used as an electrostatic stabilizer with hydroxypropyl methylcellulose (HPMC) as a steric stabilizer and sucrose to suppress the hydration of the polymer matrix to accelerate drug release using the wet-milling method. The formulated ALV-NC showed a small and uniform particle size with a marked increase in dissolution in different dissolution media compared with the marketed product (Entereg®). The formulation and preparation processes were further refined by a 3^2 factorial design with SDS content (X_1) and HPMC content (X_2) as independent factors and particle size, *in-vitro* release (IVR) at 30 min., and zeta potential as variables for responses. The optimal condition was determined as a combination of 1.07 mg per capsule of SLS and 0.31 mg per capsule of HPMC. The particle size (D 90), IVR at 30 min., and zeta potential were all determined to be (Y_1) 472 ±12.57 nm, (Y_2) 100.4 %, and (Y_3) -39.1 mV, respectively. The optimized formulation showed irregular shape morphology as observed by scanning electron microscopy (SEM) and crystallinity, along with molecular interaction between drug and stabilizer determined by differential scanning calorimetry (DSC), X-ray powder diffraction (PXRD), and Fourier transform infrared spectroscopy (FT-IR). Furthermore, the in vivo pharmacokinetics of the formulated ALV-NC were evaluated in Sprague-Dawley rats by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). The results indicated that the C_{max} and AUC_{0-24} of ALV-NC were 2.38-fold and 1.74-fold higher

than those of the marketed capsule formulation (Entereg®), respectively. The screening and formulation optimization procedures used in this study are a practically viable method for increasing the oral bioavailability of ALV.

Keywords: Nanocrystals, Alvimopan, Bioavailability, Experimental design, wet milling, Fluidized bed processing.

INTRODUCTION

The enormous array of prospective drug candidates generated by cutting-edge high throughput screening and combinatorial chemistry, which has greater biological selectivity and specificity with target receptors. But because of these physical and chemical properties, drugs in the pharmaceutical pipeline are often less water-soluble. More than 40% of chemical medications are thought to be poorly water-soluble, which could lead to inconsistent and low oral bioavailability. Poor aqueous solubility frequently results in low oral bioavailability since it is necessary for drug aqueous medium dissolution in the GI to occur after oral absorption [1]. With lead compounds or therapeutic candidates, the majority of pharmaceutical companies are dealing with all these issues. Many strategies, such as salt or prodrug synthesis, the use of co-solvents, cyclodextrin complexation, lipid-based formulations, microemulsions, micellar systems, amorphization, and nanotechnology, have been developed to increase the solubility and dissolution rate of these weakly soluble medications [2].

The nanocrystals technique has emerged as one of the most promising methods among those listed above. difficulties with inadequate aqueous solubility and raising the bioavailability of several medications One popular method uses nanocrystals [3]. Drug particle nanocrystals, which are typically sized between 200 to 500 nm, are stabilised by surface stabilisers and have a size less than 1 µm. Depending on its intended use, the final product's ideal particle size may change [4]. Because size reduction of drug crystals enhances their specific surface area and, in turn, their bioavailability, nanocrystals are distinguished by a rapid dissolving rate and high solubility [5]. By enhancing the dissolution rate defined by the Noyes-Whitney equation, the apparent solubility governed by the Kelvin-Ostwald-Freundlich equation, and mucoadhesion by virtue of reduced particle size, nanocrystals provide advantages for biopharmaceuticals in in vitro and in vivo performance investigations [6]. However, ultrafine particles, particularly those smaller than 100 nm, exhibit an increase in saturation solubility, which also accelerates the dissolution rate. Hence, to boost saturation solubility, the nanocrystal technique depends on smaller crystals with a larger specific surface area and a smaller extent [7]. Drug nanocrystal preparation methods have been divided into bottom-up and top-down categories based on how the nanoparticles are formed. Due to the multiple limitations of the bottom-up method, such as the difficulties in crystal management and the potential organic solvent residues involved in the anti-solvent precipitation procedures, the top-down method is the main choice for commercial manufacture of medicine nanocrystals. Top-down processes, such as micro fluidization, high pressure homogenization, and wet media milling, use mechanical attrition to break down big

particles into nanoparticles [8]. Wet media milling is the most effective method for creating drug nanocrystals; during milling, coarse drug particle size reduction is accomplished by collision, impaction caused by the movement of milling beads, and shear force generation. Wet media milling has resulted in the commercialization of numerous nanocrystal formulations, including Rapamune (Sirolimus, Wyeth), Emend (Aprepitant, Merck), Megace (megestrol acetate, Par Pharmaceutical), and TriCor (fenofibrate, Abbott) [4]. Due to a large increase in surface area, nanocrystal technology has been shown to boost the solubility and subsequently the bioavailability of insoluble medications. This technology was assessed by research scientists and provided in this research article.

Alvimopan (Entereg®), an oral and peripherally acting μ—opioid receptor (PAM-OR) antagonist, was authorized by the FDA in May 2008 as a single-line medication for the management of post-operative intestinal blockage following bowel resection with primary anastomosis. It has a high molecular zwitterion (MW 425, cLog P = 2.2, cLogDpH 6.5 = 0.9) because of these properties, which help to limit solubility and cause limited absorption as well as permeability. Additionally, although gut microflora metabolism lowers systemic exposure, amide hydrolysis results in peripherally exposed active metabolites [9]. Alvimopan aids patients in having their first bowel movement, hastening the digestive recovery. Alvimopan has a bioavailability of only 6% despite having satisfactory clinical benefits, which is primarily due to API's poor solubility as a BCS Class IV molecule. The USFDA recommended that the brand formulation only be administered during hospitalization so the patients can be monitored for cardiac issues, if any, because during the clinical studies of the brand product (Entereg®), there were observations of myocardial infarction in a few patients. As a result, under its programme of Risk Evaluation and Mitigation Strategy (REMS), the USFDA's recommendation was made. It is clear from this that Alvimopan causes cardiac toxicity. The reported side effects of Entereg® include dyspepsia, stomach pain, nausea, vomiting, and diarrhea in addition to myocardial infarction.

Many products have recently been released on the market with the goal of increasing API's solubility and bioavailability through the use of nanocrystals. It is a low-cost and simple to use technique, hence many formulation scientists are exploring it extensively for water-insoluble medicines for oral delivery [10].

In this study, a stable ALV nanocrystalline solid dispersion was made by combining the wet milling method with a fluidized bed processor. Based on the aforementioned information, it was determined that Alvimopan needed to be reformulated in order to provide improved solubility and increased bioavailability. Due to Alvimopan’ s increased bioavailability, it may be possible to use a lower dosage than is currently recommended while maintaining an effective pharmacokinetic profile. Additionally, a lower dose may reduce the likelihood of side effects, enhancing patient safety.

MATERIALS & METHODS

Materials

Alvimopan Powder was received from MSN Lifesciences Pvt. Ltd., Chandampet, Telangana, India. Hydroxypropyl methylcellulose (Methocel E5 LV) was obtained from Dow chemicals, Switzerland. Sodium lauryl sulphate was acquired from Rankem, Mumbai, India. Sucrose was purchased from Signet excipients Mumbai, India. SuperTab 21 AN was purchased from DFE Pharma, Cuddalore, Tamilnadu, India. Yttria stabilized zirconium oxide beads were obtained from NETZSCH Vakumix GmbH. Analytical grade chemicals were purchased from Merck, Mumbai, India and from other renowned vendors and used as received without further alteration.

Method of Preparation

In our earlier screening investigations, we assessed a number of surfactant types (including Polysorbate 80, Poloxamer 188, and SLS) to determine wettability with ALV. In a nutshell, 1.06 mg of SLS was dissolved in deionized water while being stirred magnetically, and the pH of the surfactant solution was measured at 5.20 (25°C). After stirring for 10 minutes, 12 mg of bulk ALV powder per capsule was dissolved in the aforementioned solution. After that, the mixture was put into the NETZSCH Delta Vita ® 15-300 (Nano mill) with zirconium oxide milling beads that were 0.3 mm in size as the milling media and took up 70% by volume of the milling chamber. The following parameters were used for the milling procedure: agitator speed of 3000 rpm, milling rotation speed of 75 rpm, and milling time of 60 min.

After the milling was done, a solution of HPMC and sucrose was added to the slurry and mixed for 10 minutes with a lot of force. Then, using a fluidized bed processor (GPCG 1.1 and ACG), the prepared formulation was adsorbed onto an inert carrier, SuperTab 21 AN, to make a dried powder. The resulting powder was then placed inside a size "01" firm gelatin capsule.

Experimental design and optimization

For making an Alvimopan nanocrystal formulation, preliminary tests were done with different formulation and process variables, such as the amount of SLS and HPMC per capsule (mg), the size of the beads, the speed of wet milling, and the speed of recirculation. The key factors that affected the particle size, in vitro drug release, and zeta potential of the nano crystal formulation were found to be the amounts of SLS and HPMC per capsule (mg). In order to optimize the variables, a 3^2 randomized complete factorial design was utilized. Particle size, in-vitro drug release at 30 minutes in pH 4.5 acetate buffer dissolving media, and zeta potential of the produced formulations were investigated systematically in relation to the effects of these two crucial formulation variables. The experimental range was chosen for each factor. In this design, two variables—the content of SLS and content of HPMC per capsule—were assessed at three different levels: low, medium, and high.

The independent factors and their levels are listed in **Table 1.** As indicated in **Table 2,** nine experimental runs were produced and thoroughly assessed using the CQA (response variables) of

particle size (Y1), zeta potential (Y2), and in vitro release at 30 min. Data processing and the creation of response surface plots were done using Design Expert® software (version 12.0.1.0, Stat-Ease Inc., Minneapolis, MN).

Table 1: 3^2 randomized full factorial design parameters and experimental condition

Independent Variables	**Levels**		
	Low (-1)	Medium (0)	High (+1)
X_1: Content of SLS per capsule (mg)	0.3	0.75	1.2
X_2: Content of HPMC per capsule (mg)	0.3	0.6	0.9
Dependent Variables			
Y_1: Particle size D_{90} (nm)			
Y_2: Drug release at 30 min (%)			
Y_3: Zeta potential (mV)			

The experimentally observed values of the responses were quantitatively compared with expected values to confirm the experimental design. The relative error (%) was determined using the following equation:

Relative error (%) = {(Predicted Value−Experiment value)/Predicted value} X 100

Characterization of ALV Nano Crystals

Particle size, Zeta potential & Polydispersity index

Before testing, ALV-NC samples were thoroughly dissolved in deionized water and diluted to about 20 µL in 5 mL. The particle size, zeta potential, and PDI of ALV nanosuspension were determined by dynamic light scattering (Zetasizer ZEN3600, Malvern Ltd., UK) (Zetasizer ZEN3600, Malvern Ltd., UK). Each sample was measured three times.

Particle morphology

A scanning electron microscope was used to examine the morphology of Bulk ALV powder and optimized ALV-NC solutions. Before being observed, a thin layer of gold-palladium was sputter deposited onto each sample and it was then attached to an aluminium stub using double-sided tape. The samples underwent examination at a 20 KV accelerating voltage.

Differential Scanning Calorimetry (DSC)

A DSC 250 system was used to measure the thermograms of bulk ALV powder, excipients (SLS, HPMC, sucrose, and SuperTab 21 AN), physical mixture (PM), and ALV-NC DSC (TA Instruments, New Castle, DE, US). Each sample was accurately weighed and put into an aluminium pan with a punctured lid (2.0 mg). A nitrogen gas flow rate of 50ml/min was employed with a heating rate of 40–250°C at 10°C/min.

Weighed and put into a slandered aluminium sample holder with a hermetic lid were 2.0 mg of alvimopan dihydrate. The nitrogen gas flow was 50 ml per minute, and the sample holder's temperature was initially kept at 40 °C before being elevated to 250 °C at a rate of 10 °C per minute. DSC was precalibrated for the baseline using an empty pan.

X-ray diffraction of powder (PXRD)

Powder X-ray diffractometers (Malvern Panalytical, Aeries) were used to assess the crystallinity and crystal structure variation of bulk ALV powder, excipients (SLS, HPMC, sucrose, and SuperTab 21 AN), PM, and ALV-NC using Cu K radiation (30 KV and 30 mA). The diffraction patterns were scanned from 2 to 40° (2θ) with a step size of 0.043°C. The final formulation's nanocrystals were isolated and put to the test.

Fourier transform infrared spectroscopy (FT-IR)

An FTIR spectrometer was used to record the FTIR spectra of bulk ALV powder, excipients (SLS, HPMC, sucrose, and SuperTab 21 AN), PM, and ALV-NC in the region of 4000 to 400 cm-1 with a resolution of 1.0 cm-1 (Perkin Elmer 2 spectrometer).

Analysis of ALV by High-Performance Liquid Chromatography (HPLC)

An HPLC (high-performance liquid chromatography) device from Waters model e2695 was used to measure the ALV concentration (Milford, MA, USA). At 30°C, 20 µL of material was injected onto an analytical column made of Zorbax SB C8 (3.5m; 4.6 x 150 mm, Agilent, Santa Clara, CA, USA). As the mobile phase, 0.2% orthophosphoric acid in acetonitrile was pumped at a ratio of 80:20 v/v at a flow rate of 1.0 mL/min. a gradient elution system with a 210 nm detection wavelength. ALV standard curve was used to calculate ALV concentration. Each sample was measured in triplicate.

Study of *in-vitro* dissolution

The dissolution of bulk ALV powder, PM, ALV-NC, and the commercial product Entereg® was studied using the USP Apparatus 2 (Distek Inc., USA, North Brunswick) and Japanese sinkers. The paddle speed was adjusted to 50 rpm and the temperature to 37°C (0.5°C). Acetate buffer was used as the dissolving medium (pH 4.5). The vessels containing the samples (equivalent to 12 mg ALV) were filled with the dissolution medium in 900 mL. At intervals of 5, 10, 20, 30, 45, 60, 90, and 120 minutes, 10 mL of the dissolving medium were removed and replaced with an equal volume of brand-new dissolution media. In order to assess the content of ALV by HPLC analysis, the collected sample was filtered through a 0.45 µ PVDF syringe filter using clear solution after discarding 3 mL of volume.

Oral Bioavailability In-*vivo*

A pharmacokinetic study was done with male Sprague-Dawley rats that weighed 200 ±20 g. The three groups—bulk ALV powder (Control group), ALV-NC, and commercial formulation RLD

(Entereg®)—each contained 12 rats. The rats fasted for 12 hours prior to the experiment but had free access to water. ALV doses of 30 mg/kg body weight were given orally for ALV-API, ALV-NC, and RLD. At the predetermined intervals of 30 min, 1, 2, 3, 7, 16, and 24 hr., 0.5 mL of blood were drawn from the retroorbital plexus and placed in a heparinized tube. The blood sample was centrifuged at 4000 g for 5 minutes to separate the plasma, which was then kept at -20 °C for later analysis. Plasma is extracted with the aid of solid-phase extraction cartridges. Using high-performance liquid chromatography, samples were examined (HPLC).

RESULTS AND DISCUSSION

In this study, the performance of ionic and non-ionic surfactants was investigated and evaluated. It was found that ionic surfactants containing sodium lauryl sulphate had better wetting and stabilizing abilities than non-ionic surfactants such as polysorbate 80, polysorbate 20, Poloxamer 407, and Poloxamer 188. Drug particles that are nanosized have a significant increase in surface area, which increases Gibbs-free energy and is detrimental to maintaining product stability [11]. In order to ensure acceptable stability of ALV-NC, a combination of polymeric and ionic surfactant was used as the stabilizer. Hydroxypropyl methylcellulose (HPMC) was selected as a steric stabilizer because of the unique advantages of stabilizing nanocrystals [6]. Additionally, the HPMC matrix's behavior when combined with a solute with a high concentration, like sucrose, showed an impact on the removal of the gel layer during dissolution.

Optimization of ALV-NC using 3^2 randomized full factorial design

Stabilizers are crucial in nanocrystal formulations because they maintain system stability and stop medication particles from disintegrating. On the other hand, wet media milling's ability to produce high-quality nanocrystals is only somewhat influenced by the stabilizer concentration. The right amount of stabilization is essential when creating nanocrystal formulations because insufficient amounts are incapable of maintaining system stabilization and excessive amounts, given their higher solubility and viscous nature, have detrimental effects on nanocrystal formation and stability [7]. In addition, it is discovered that mixes of various stabilizers, such as ionic surfactants and polymers, have a combined influence on nanocrystal stability [12]. The aforementioned two criteria showed a considerable influence on the ALV-NC formulation in the earliest research for its preparation. Hence, a 32 randomized complete factorial design was used to determine the essential elements for optimization, which were the concentrations of SLS and HPMC in each capsule. In this investigation, there were nine experimental runs. **Table 2** summarizes the outcomes of the selected responses for all experiments, including particle size (D90), zeta potential, and in vitro release in 30 min.

Table 2: 3^2 full factorial design with observed response values of Alvimopan nanocrystal formulation.

Batch No.	Content of SLS (mg) (X_1)	Content of HPMC (mg) (X_2)	Particle size D90 (nm) (Y_1)	Drug release at 30 min. (%) (Y_2)	Zeta potential (mV) (Y_3)
AVP-12-008	**0.75**	**0.3**	655	98	-34.3
AVP-12-009	**0.3**	**0.3**	1490	93.9	-33.6
AVP-12-010	**0.75**	**0.6**	538	97.7	-27.8
AVP-12-011	**1.2**	**0.9**	823	89.4	-37.3
AVP-12-012	**0.3**	**0.9**	1020	93	-21.9
AVP-12-013	**1.2**	**0.6**	676	97.4	-37.5
AVP-12-014	**0.3**	**0.6**	1250	101.9	-21.1
AVP-12-015	**0.75**	**0.9**	662	106.3	-33.6
AVP-12-016	**1.2**	**0.3**	529	101.8	-37.7

ANOVA was used to assess the importance of the variable effects and model selection for response analysis as shown in **Table 3**. The independent factors X1 and X2 were treated as A and B, respectively, in the model. The independent factors content of SLS per capsule (A) and content of HPMC per capsule (B) were found significant for particle size D90 and zeta potential. The link between them was found to be best described by the quadratic model. Yet, it was determined that it had no bearing on the drug release after 30 minutes. Polynomial equations characterizing the quantitative effects of independent components and their interactions on the outcome were produced after a multivariate linear regression analysis of the data.

PSD D_{90} = + 2893.92593 - 4055.80247 * Content of SLS/capsule - 1712.77778 * Content of HPMC/capsule + 1414.81481 * Content of SLS/capsule * Content of HPMC/capsule + 1710.28807 * (Content of SLS/capsule) ^{2} + 464.81481 * (Content of HPMC/capsule) ^{2} (1)

Drug release at 30 min = + 89.85000 + 12.70370 * Content of SLS/capsule + 13.19444 * Content of HPMC/capsule - 21.29630 * Content of SLS/capsule * Content of HPMC/capsule (2)

Zeta potential = - 25. 93889 - 13.29630 * Content of SLS/capsule + 7.11111 * Content of HPMC/capsule (3)

A positive sign indicates that a factor improves the response, whereas a negative sign indicates that the relationship between the factor and the response is inverted. A 3D response surface plot depicted in **Figure 1** illustrates how a factor affects a response. Learn more about the interrelationship between components A and B by using this plot.

To understand how the various parameters affected the CQAs, the 3D surface plots were employed.

Figure 1 illustrates the effects of interactions between various formulations and process parameters on ALV-particle NC's size (Y1, Figure 1A), drug release at 30 minutes (Y2, Figure 1B), and zeta potential (Y3, Figure 1C).

While applying higher concentrations of SLS and HPMC effectively reduces the particle size in the case of Y1, this is probably due to the increased polymer supplementation needed to maintain optimal stabilization in the presence of an ionic stabilizer, as seen in **Figure 1A**. However, the modest increase in ALV-NC particle size was brought on by greater polymer concentrations combined with low SLS concentrations.

The drug release profile at 30 minutes was clearly shown to be less affected by increasing the concentration of SLS and HPMC in the case of Y2 in **Figure 1B.**

As can be seen in **Figure 1C**, in the case of Y3, it demonstrated that raising the ionic stabilizer concentration raises the zeta potential of the ALV-NC formulation. Conversely, the zeta potential was found to decrease as polymer concentration increased [13].

Table 3: Analysis of variance (ANOVA) quadratic statistical model for Particle size D_{90} (Y_1)

Source	Particle size D_{90} (Y_1) Quadratic					Drug release at 30 min. (Y_2) 2FI					Zeta potential (Y_3) (Linear) linear				
	Sum of Squares	df	Mean Square	F-value	p-value	Sum of Squares	df	Mean Square	F-value	p-value	Sum of Squares	df	Mean Square	F-value	p-value
Model	8.940E+05	5	1.788E+05	58.28	0.0035	37.24	3	12.41	0.3511	0.7909	242.11	2	121.05	7.53	0.0231
A-SLS Conc.	5.000E+05	1	5.000E+05	162.94	0.0010	0.0067	1	0.0067	0.0002	0.9896	214.80	1	214.80	13.36	0.0106
B-HPMC Conc.	4760.17	1	4760.17	1.55	0.3014	4.17	1	4.17	0.1179	0.7453	27.31	1	27.31	1.70	0.2402
AB	1.459E+05	1	1.459E+05	47.56	0.0062	33.06	1	33.06	0.9352	0.3779	-	-	-	-	-
A^2	2.399E+05	1	2.399E+05	78.18	0.0030	-	-	-	-	-	-	-	-	-	-
B^2	3500.06	1	3500.06	1.14	0.3638	-	-	-	-	-	-	-	-	-	-
Residual	9205.11	3	3068.37	-	-	176.77	5	35.35	-	-	96.45	6	16.08	-	-
Cor Total	9.033E+05	8	-	-	-	214.01	8	-	-	-	338.56	8	-	-	-

Note: df – Degree of Freedom.

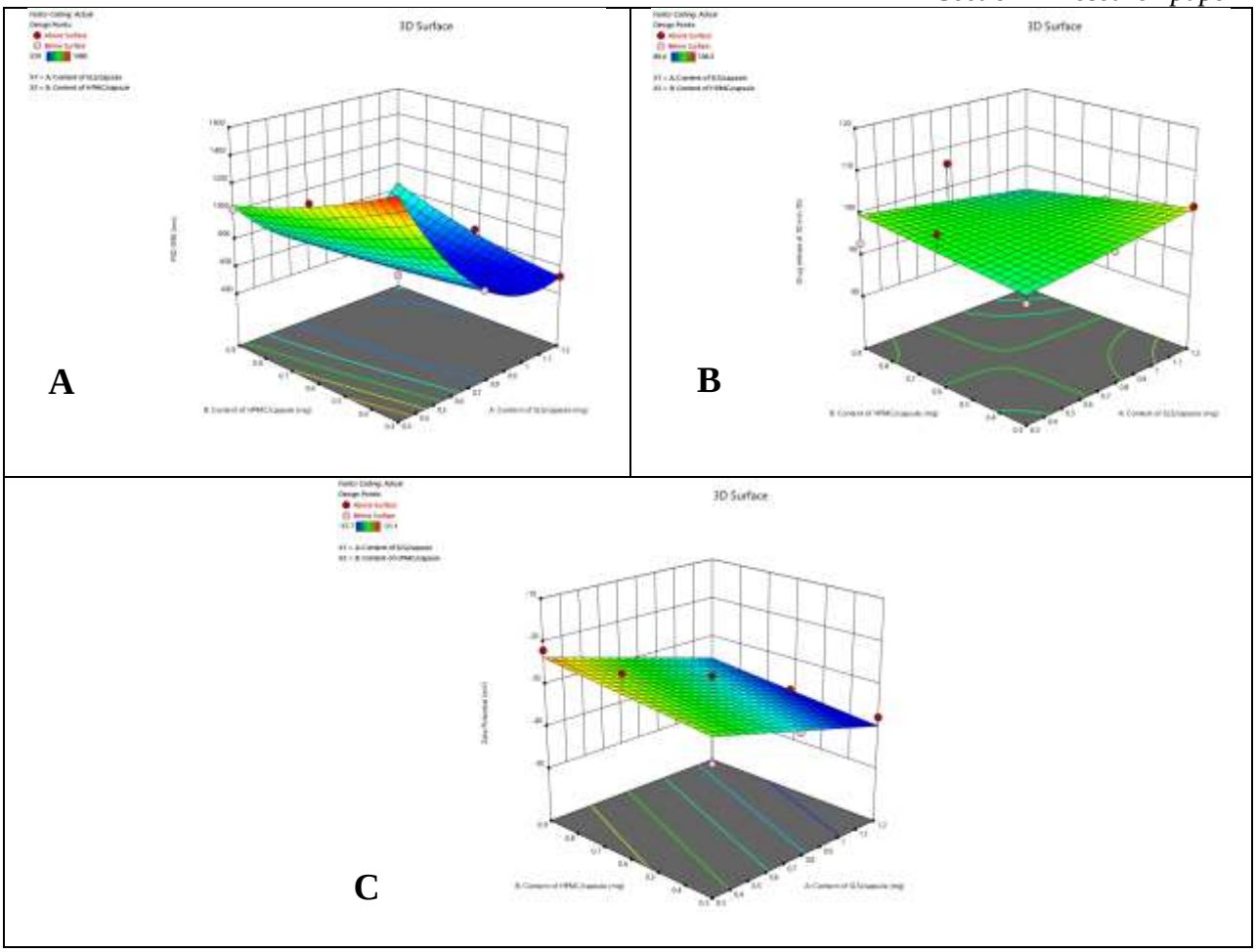

Figure 1. (A) 3D response surface plot representing interpretation of independent variables on particle size D_{90}. **(B)** 3D response surface plot showing interpretation of independent variables on drug release at 30 min. **(C)** Response surface showing interpretation of independent variables on zeta potential.

By comparing observed values with the predicted responses, it was possible to verify the correctness of the optimized formula. The experimental results were found to be within the range of the expected values, as shown in **Table 4**. The relative error percentage between anticipated and observed results was determined to be less than 5%.

Table 4: Optimized formulation composition and predicted vs. observed response values.

Optimized Formulation	Optimized concentration	Responses	Predicted Value	Observed value	% Relative Error
Content of SLS per	1.07	Particle size D_{90} (nm)	495.67	472	4.78

capsule (mg)					
Content of HPMC per capsule (mg)	0.31	Drug release at 30 min. (%)	100.42	100.4	0.02
		Zeta potential (mV)	-37.91	-39.1	-3.14

In order to optimize the Alvimopan nanocrystal formulation, a 32-way randomized full factorial design was verified.

Zeta potential, particle size, and the poly dispersibility index (PDI)

The final formulation used in this investigation contained 12 mg of ALV per capsule, 0.31 mg of HPMC per capsule, and 1.07 mg of SLS per capsule, all of which were dissolved in 5 mL of deionized water. ALV-average NC's particle size was 472 ± 12.57 nm. The ALV-PDI NC's score was 0.191 ± 0.0076, indicating good particle homogeneity [14]. More than 20 mV of zeta potential was required to stabilize the formulation [15]. The formed ALV-NC had a zeta potential of -39.1 ± 0.87 mV, which guaranteed the stability of the ALV-NC slurry. The values for the zeta potential, PDI, particle size of reconstituted ALV-NC was -22.4 ± 0.64 mV ,593± 24.78 nm and 0.219 ±0.0049, respectively. These findings show that the fluidized bed processor's dry adsorbed slurry was effectively mixed with water.

Particle morphology

The surface morphology of bulk ALV and ALV-NC is shown in **Figures 2 and 3,** respectively. Bulk ALV powder showed flaky shape with very large particle size, while ALV-NC showed continuous and reduced particle size flaky shape particles to below 1 micron. It was observed that after milling, there is no change in particle shape.

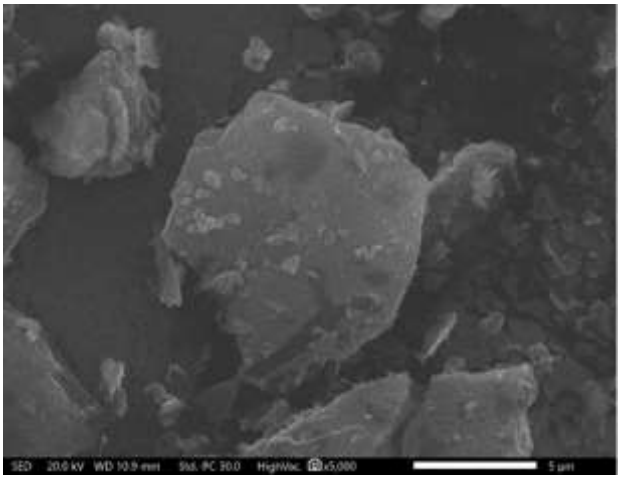

Figure 2. SEM of Bulk ALV powder

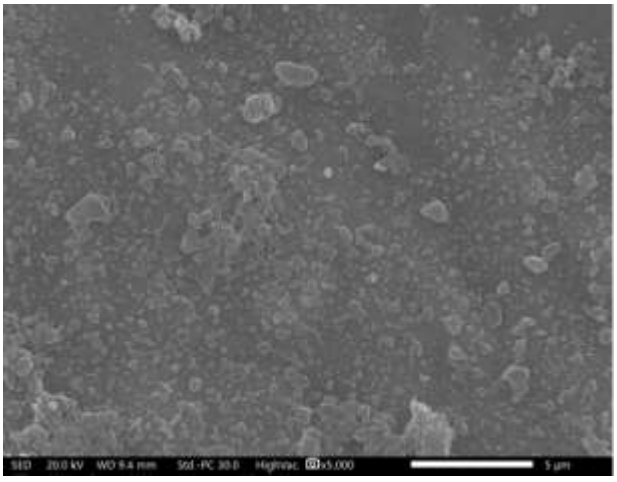

Figure 3. SEM of ALV-NC

DSC

The polymorphic character and crystalline state of the ALV in nanocrystalline form were examined using DSC. **Figure 4** displays the DSC thermograms of bulk ALV powder, HPMC, SLS, sucrose, SuperTab 21 AN, PM, and ALV-NC. ALV-PM peaks at 223 °C and 185 °C, which are similar to the melting peak of bulk ALV powder but have a higher melting temperature, which may be because of the presence of stabilizers in the formulation. Bulk ALV powder displayed a classic endothermic melting peak at 212 °C. With a higher melting point peak of 220 °C and a weaker peak intensity than bulk ALV powder and ALV-PM, ALV-NC displayed slightly different DSC thermograms. The development of crystal defects and lattice disorders during the milling-drying process may be to responsible for this variation in peak position and melting temperature [16], [17]. Most likely, this is because of how drugs mix with excipients and the size of the particles.

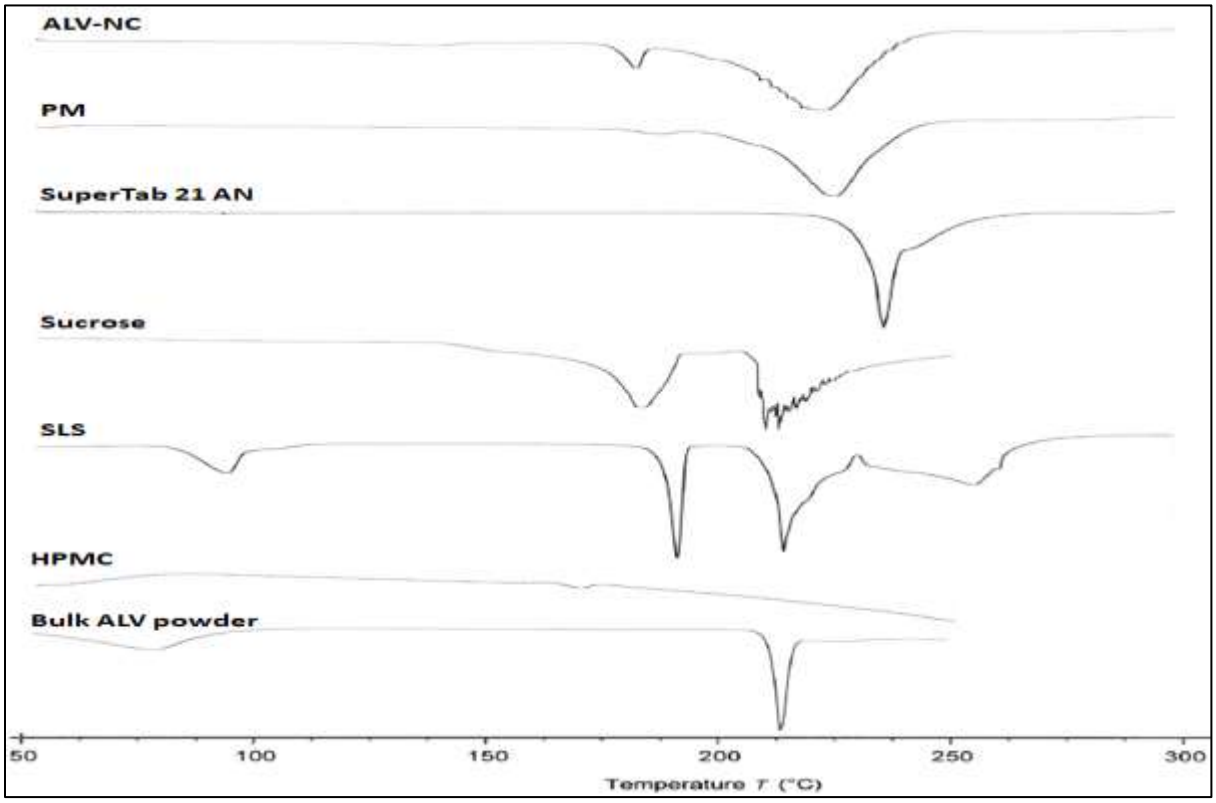

Figure 4. DSC images of bulk ALV powder, HPMC, SLS, Sucrose, SuperTab 21 AN, PM, and ALV-NC.

PXRD

PXRD analysis, whose X-ray diffractogram is displayed in **Figure 5**, was carried out to further confirm the crystalline state of various samples. For a bulk ALV powder at the 2Θ values of 8.2653, 11.9782, 14.2434, 15.5186, 17.8072, 19.2833, 20.9908, 24.7507, and 25.9425,

characteristic strong peaks were seen. Moreover, physical mixture (PM) was used to produce the distinctive diffraction peaks of bulk ALV powder, demonstrating that the mixing method had no effect on the crystalline structure of ALV. The most distinct crystalline peaks in ALV-NC, however, were less intense and had a rougher diffraction pattern than in bulk ALV and PM. This may have been due to the excipients' distribution of the ALV and the finer particle size that resulted from milling the ALV [18]. In spite of the fact that some of the crystalline ALV for coexisting systems was converted into an amorphous state, these PXRD results were matched to DSC studies, and it was shown that the crystalline structure was retained following milling-fluidized bed drying [19].

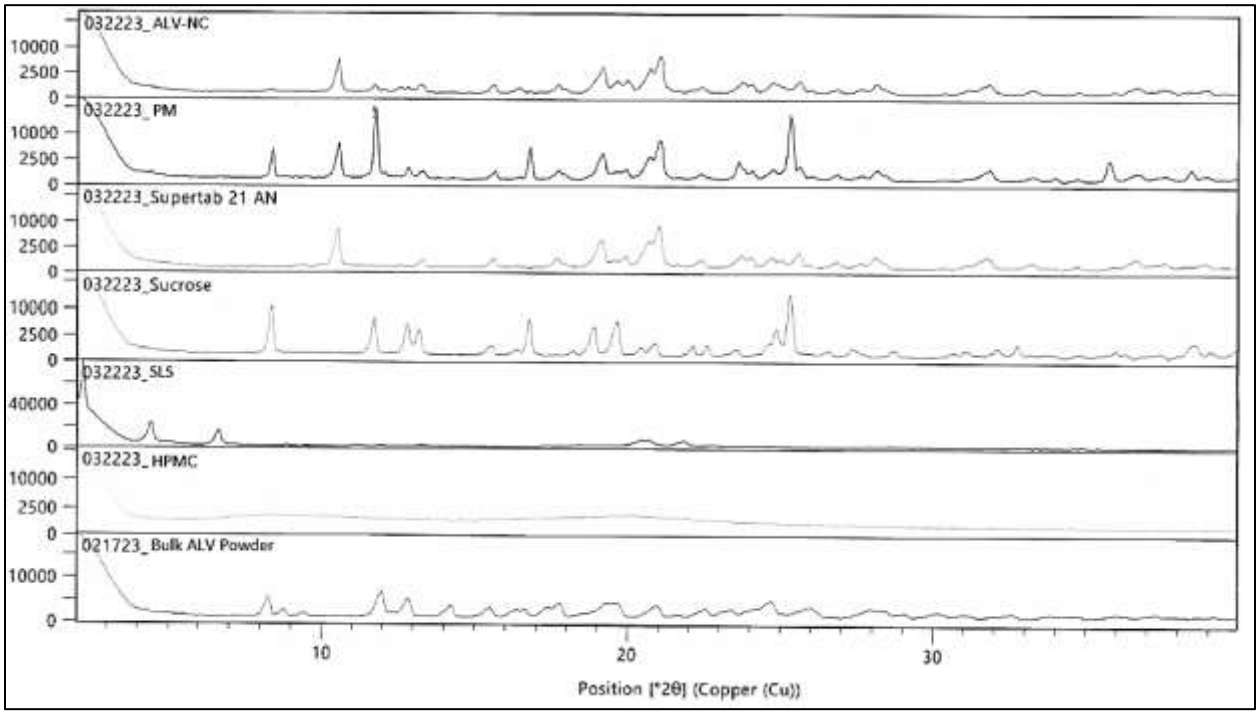

Figure 5. PXRD patterns of Bulk ALV powder, HPMC, SLS, Sucrose, SuperTab 21 AN, PM, and ALV-NC

FT-IR Analysis

The possibility of a molecular interaction between stabilizer and medications was investigated using FT-IR spectroscopy.

Figure 6 displays the FT-IR spectra of bulk ALV powder, HPMC, SLS, sucrose, SuperTab 21 AN, PM, and ALV-NC. The FTIR spectra of the bulk ALV powder revealed large bands at 3410 cm^{-1} for the carboxylic acid group's OH, 3207 cm^{-1} for amidic NH, 1681 cm^{-1} for the carboxylic acid group's carbonyl group, and 1587 cm^{-1} for the amide group's carbonyl group [20] . Both PM and ALV-NC recognized all typical ALV peaks. The majority of the ALV peaks were also maintained, showing that there was no detectable interaction between the functional groups of

ALV and stabilizers as well as good compatibility between drugs and other excipients used in formulation. Additionally, there was no obvious shift in wave number or other significant disappearance in the spectrum.

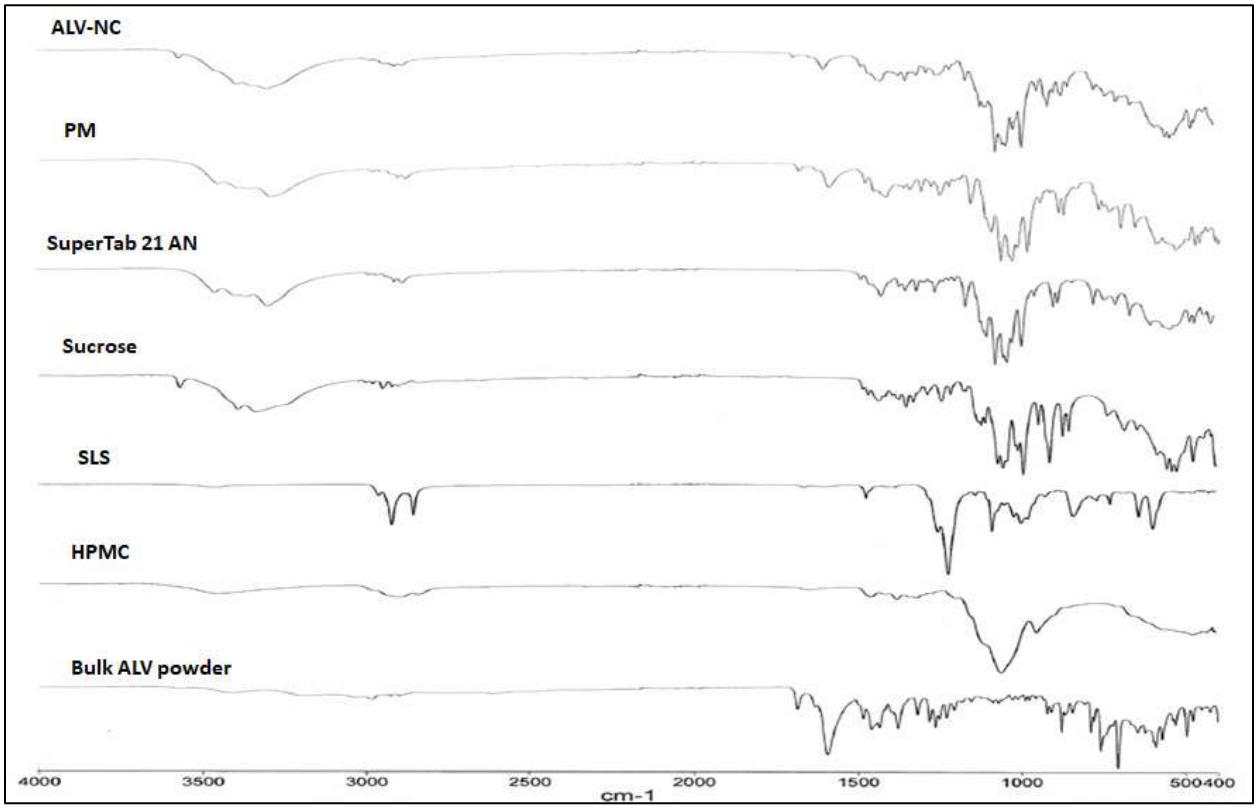

Figure 6. FT-IR spectra of Bulk ALV powder, HPMC, SLS, Sucrose, SuperTab 21 AN, PM, and ALV-NC

Study on In-*vitro* dissolution

Figure 7 depicts the dissolving profile of bulk ALV powder, PMs, and ALV-NC in a pH 4.5 acetate buffer dissolution media. While PM demonstrated a small increase in the dissolution profile with time compared to bulk ALV powder, the accumulated dissolution was still only 68% after 90 minutes. Bulk ALV powder was more difficult to dissolve. The dissolving profile and particle wettability are both enhanced by excipients. When it came to ALV-NC powder, the rate of dissolution was much faster. More than 70% of the drug in the ALV-NC powder was released in the first 5 minutes, and about 95% was released in the first 60 minutes. In the pH 4.5 acetate buffer, the similarity factor f2 for the dissolution profiles of ALV-NC and bulk ALV powder was only 6.20, and f2 was likewise less than 20 for ALV-NC and PMs. These findings show that ALV-NC was significantly superior to bulk ALV powder and PMs. The improvement in the dissolving rate of ALV-NC was therefore inferred to be the result of a decrease in the particle size range to the nonorange and an increase in the surface area of nanoparticles.

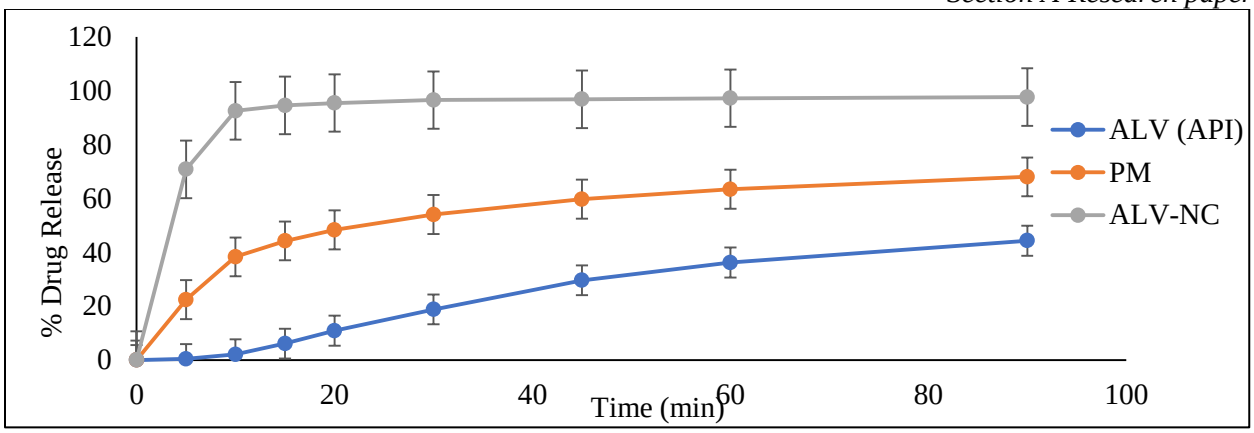

Figure 7. Comparative Dissolution of Bulk ALV powder, PM & ALV-NC in 4.5 pH Acetate Buffer.

Study on *In-vivo* Pharmacokinetics

In Sprague-Dawley rats, the in vivo pharmacokinetics of bulk ALV powder, ALV-NC, and the marketed capsule formulation (Entereg®) were investigated and compared. **Figure 8** displays the plotted ALV plasma concentration-time curves, and **Table 5** provides an overview of the key pharmacokinetic characteristics. As demonstrated, ALV-NC had an area under the curve (AUC_{0-24}) and maximum drug concentration in plasma (Cmax) that were approximately 2.67 and 2.24 times higher than those of bulk ALV, respectively. The Cmax and (AUC_{0-24}) of ALV-NC, however, increased by roughly 2.38 and 1.74 times more, respectively, in comparison to the marketed capsule formulation. ALV-NC had a relative bioavailability of 224.64 % compared to bulk ALV powder and 174.63% compared to marketed capsule formulation, respectively. This result is consistent with in vitro dissolution findings. The ALV-NC formulation significantly increased the oral bioavailability of ALV in rats, as shown by the reported pharmacokinetic data.

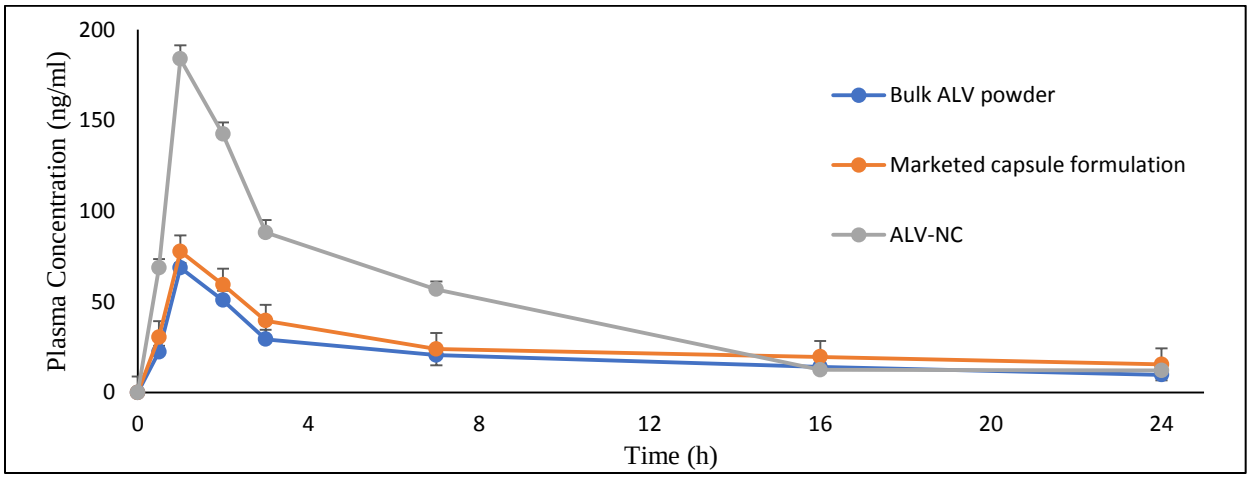

Figure 8. ALV plasma-time curves in rats after oral administration of Bulk ALV powder, Marketed capsule formulation & ALV-NC (mean ± SD, n=4)

Table 5 Pharmacokinetic parameters after single oral administration of ALV formulations to rats at a dose of 30 mg/kg (mean ± SD, n=4)

Sr. No.	Parameters	Bulk ALV powder	Marketed capsule formulation	ALV-NC
1.	C_{max} (ng/ml)	68.80 ± 2.6	77.8 ± 7.19	183.84 ± 7.15
2.	T_{max} (h)	1	1	1
3.	$t_{1/2}$ (h)	17.7 ± 2.71	29.42 ± 5.84	6.705 ± 0.26
4.	$AUC_{0\text{-}24h}$ (ng h/ml)	479.51 ± 49.88	616.37 ± 62.67	1059.60 ± 12.01
5.	$AUC_{0\text{-}\infty}$ (ng h/ml)	722.96 ± 33.40	1316.39 ± 270.68	1167.14± 10.53

According to the literature papers that are currently available, no experiments have been done recently to increase the bioavailability of ALV. The smaller particle size and higher surface area of ALV-NC increased its in vitro dissolving rate, which is what caused its improved oral bioavailability [21]. Additionally, nanoparticles can significantly increase membrane permeation and the adhesion surface area between intestinal epithelium membrane and nanoparticles when the ALV-NC enters the digestive tract [22].

CONCLUSION

This study reveals that nanocrystal technology is a promising technological platform for overcoming the problem of the low oral bioavailability and low solubility of BCS Class IV medications. The goal of the current work was to increase the bioavailability of the medicine ALV powder by using a top-down method of nano-crystallization. SLS and HPMC were used as stabilizers during the effective preparation of ALV-NC using the wet milling technique. Zeta potential, PDI, and average particle size were all -39.1 ± 0.87 mV, 0.191± 0.0076, and 472 ± 12.57 nm, respectively. Furthermore, physicochemical analysis of ALV-NC revealed that during the milling-drying process, some of the crystalline ALV changed into an amorphous condition. The medicine and other excipients employed in formulation were found to be well compatible according to FTIR data, which also showed that there was no discernible interaction between the functional groups of ALV and stabilizers. The optimized formulation's scanning electron microscopy revealed that, aside from differences in particle size, the morphology of bulk ALV powder and its nanocrystal form is identical. These physical data allow us to infer that Alvimopan' s physicochemical characteristics and stability would remain unchanged even after the nano crystallization procedure. ALV-NC considerably improved in vitro dissolution as compared to bulk ALV-powder and ALV-PM. ALV-NC in rats had a significantly increased bioavailability, according to the later pharmacokinetic investigation. There is a strong possibility that the dose of ALV can be decreased while still achieving an equivalent pharmacokinetic profile at the dose that is currently approved due to the improved bioavailability. Moreover, a

lower dose may lessen the likelihood of side effects, enhancing patient safety. The creation of ALV nanocrystal formulations is a straightforward, repeatable, and environmentally benign process that does not require the use of toxic chemical solvents to create the nanocrystals. In conclusion, the ALV-NC developed in this work may be an effective method for increasing the oral bioavailability and dissolution of ALV.

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CONFLICT OF INTEREST

The authors say they have no conflict of interests.

ETHICAL APPROVALS

The institutional animal ethics committee examined and approved the study's general protocols and animal usage protocol. According to the Council for Control and Supervision of Experiments on Animals (CCSEA) criteria for animal care, all operations involving animal experimentation were carried out in accordance with their recommendations found in the Handbook for the Care & Use of Laboratory Animals.

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