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ENHANCEMENT OF FLOW PROPERTIES, SOLUBILITY, AND DISSOLUTION OF THE ATAZANAVIR BY SPHERICAL CRYSTALLIZATION

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ABSTRACT

Background: The present work aimed to develop spherical agglomerates of Atazanavir (ATZR) with enhanced flow properties, compressibility, solubility, and dissolution. 2^2 full factorial design approach was employed to develop agglomerates. **Methodology:** ATZR spherical agglomerates were prepared using bridging solvent Benzene, methanol, HPMC and evaluation for different properties. Then, ATZR immediate release (IR) capsules were formulated using spherical agglomerate, wet granulation, and direct compaction methods. **Results and discussion:** The spherical agglomerates resulted in a significant enhancement of micromeritic properties. The drug content was ranged from 91.9 % (SAA6) to 97% (SAA2). Drug content and solubility (4.88 to 39.89 mg/ml) were directly related to the concentration of HPMC and inversely related to benzene concentration ($p<0.05$). Nearly 10.12-fold enhancement in solubility of ATZR was found with spherical agglomerates. FTIR analysis demonstrated excellent compatibility between the drug and the polymer. XRD results indicated the amorphization of pure ATZR during agglomeration. The agglomerates exhibited spherical particle morphology. DSC analysis confirmed the effective encapsulation of the drug. Nearly 100% release was observed within 10 minutes from the F1 capsule formulation containing ATZR spherical agglomerates. **Conclusion:** The optimized SAA2 spherical agglomerates were utilized to manufacture immediate-release capsules via direct filling (F1). Spherical agglomerates significantly enhanced the flow properties and compressibility of the blend compared to pure ATZR. Drug release from the F1 batch was notably faster than F2 and F3 formulations. The study demonstrates the substantial improvement in flow properties, compressibility, solubility, and dissolution of ATZR using spherical agglomerates.

INTRODUCTION

With great selectivity for HIV-1 protease and potency against it, atazanavir (ATZR) is a new azapeptide protease inhibitor. The recommended dosage for patients new to therapy is 400 mg once daily. For patients already receiving treatment, it is best to take 300 mg of the medication once daily and 100 mg of ritonavir [1]. ATZR falls into BCS II, indicating that it has both low solubility and low permeability. Drugs in this class often face challenges related to their poor solubility and limited absorption in the gastrointestinal tract [2]. ATZR is characterized by its crystalline

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nature, exhibiting very low solubility of 4-5 mg/ml [3]. Furthermore, high-dose drugs often present significant challenges during formulation and manufacturing due to their inherent poor flow properties, low compressibility, and low bulk density. The high dose (300 and 400 mg) of ATZR presents significant challenges due to its poor flow properties, low compressibility, and low bulk density, making direct compression a difficult process [4].

Pharmaceutical tablet development via the direct compression (DC) process faces challenges linked to the dose and drug properties [4]. High-dose APIs pose manufacturability concerns, while low-dose drugs encounter content uniformity issues [5]. Particle engineering techniques are used for versatile platform DC tablet formulations, mitigating challenges [6]. However, strategies for high-dose APIs in DC processes are lacking. New drug compounds often suffer from limited bioavailability due to poor solubility, which can be improved by reducing API particle size [7]. Yet, smaller particles may hinder flowability. Increasing particle size is vital in tablet/capsule manufacturing, enhancing flow, solubility, dissolution, and compression properties to overcome these challenges [7, 8].

Crystal engineering is a specialized field that intentionally designs and creates molecular solid-state structures with precise characteristics and functionalities. The crystalline form, including a substance's crystal habit and polymorphic shape, significantly impacts its physical, chemical, mechanical, and biological properties [9]. Altering the crystal habit can result in different isomorphic forms, influencing particle orientation and affecting critical aspects such as powdered medications' flowability, packing, compaction, and dissolution properties. While conventional methods have long been employed for particle size enlargement, non-conventional techniques like extrusion-spherization, melt extrusion, and spherical crystallization have emerged as promising alternatives [10, 11]. The capacity of spherical crystallization to alter the physicochemical characteristics of active pharmaceutical ingredients (APIs), in particular, has drawn much interest [12]. During crystallization, this special method converts fine crystals into small, ready-to-use spherical agglomerates, improving flowability, packability, compressibility, and tabletability. Notably, it has facilitated the development of high-API-content direct compression tablet formulations and has improved drug solubility and dissolution rates for various compounds [13].

ATZR is an excellent candidate for spherical crystallization due to its poor aqueous solubility, often exceeding 100 mg per unit dose requirement, posing content uniformity and compressibility challenges in traditional manufacturing methods. Spherical crystallization addresses these issues, enhancing solubility, flow properties, and tablet manufacturability, improving drug delivery and patient compliance [14]. It generates spherical agglomerates with uniform particle size distribution, reducing the risk of segregation and improving tablet weight uniformity [15]. This versatile technique offers a valuable solution for various drug compounds, allowing tailored modifications to meet specific formulation and manufacturing needs.

Extensive analysis of the current literature has unveiled a notable scarcity of research focusing on applying spherical crystallization techniques to enhance high-dose pharmaceutical compounds' flowability, compressibility, solubility, and dissolution properties. Consequently, the primary objective of the research work was to pioneer the development of spherical crystal agglomerates for Atazanavir (ATZR), explicitly designed to surpass the performance characteristics of the original compound. These meticulously prepared spherical crystal agglomerates were subsequently subjected to form capsules, followed by a comprehensive assessment encompassing dissolution profile and a spectrum of other critical parameters.

MATERIALS AND METHODS:

Materials

J. Dunkan Healthcare Pvt Ltd Atgaon Dist. Thane gifted Atazanavir (ATZR). HPMC 5cps was obtained from Anshul Life Science Ltd Mumbai. Methanol and Benzene were taken from RUSA lab SRTMU Nanded. Lactose Monohydrate (Pharmatose 200 M), Crospovidone (Kollidon CLF), Magnesium stearate, EHG Hard Gelatin Capsules Size "0EL" and EHG Hard Gelatin Capsules Size "1" were obtained from Alkem Laboratories Ltd Mumbai.

Methods

Statistical Design of Experiments (DOE)

A 2^2-factor factorial design approach was utilized to manufacture ATZR spherical agglomerates. HPMC 5 cps (A, mg) and Benzene (B, ml) were considered independent variables, which were varied at -1 and +1 levels. Drug content (Y1) and solubility (Y2) were considered dependent variables.

The specific variables and their corresponding levels are provided in Table 1.

Table 1: Variables and levels

Variable	(-1) Low level	(+1) High level
Independent variables		
A= HPMC 5 cps	15 (mg)	30 (mg)
B= Benzene	3 (ml)	6 (ml)
Dependent variables		
Y1= Drug content		
Y2 = Solubility		

Development of ATZR spherical crystals using a bridging solvent

An accurately weighed amount of ATZR was dissolved in 100 ml of methanol (A suitable solvent) to produce a saturated solution. HPMC 5 cps polymer was dissolved in purified water and benzene as a bridging solvent. Supersaturated solutions of ATZR were separately stirred well. This super-saturated mixture was then added to 100 ml of purified water (anti-solvent/bad solvent) and stirred at 500 rpm. Then, benzene was added to this mixture with the help of a syringe, and the mixture continued stirring until crystallization occurred [16]. The details of the batches are presented in Table 2.

Table 2: Formulation batches of spherical crystals of ATZR

Batch	Factor	
	A (HPMC 5 cps) (mg)	B (Benzene) (ml)
SAA1	-1 (15)	-1 (3)
SAA2	+1(30)	-1(3)
SAA3	-1(15)	-1(3)
SAA4	-1(15)	+1(6)
SAA5	+1(30)	+1(6)
SAA6	-1(15)	+1(6)
SAA7	+1(30)	+1(6)
SAA8	+1(30)	-1(3)

Evaluation of spherical agglomerates

The spherical agglomerates were evaluated for different physicochemical properties described below.

Bulk Density

A 50 ml measuring cylinder was filled gradually with precisely weighted spherical agglomerates, and the bed was leveled without disturbing it [17, 18]. The following formula was used to get the Bulk Density (BD) from the volume, which was expressed in ml.

$$BD = \frac{Mass\ of\ sample\ in\ g}{Volume\ occupied\ by\ sample\ in\ ml}$$

Tapped density

Accurately weighted spherical agglomerates were obtained and poured into a measuring cylinder within a bulk density tester. The sample's original volume was recorded and then tapped (50–100–250 times) until there was no discernible volume change. At this point, it was recorded as the tapped volume [17, 18]. Tapped Density (TD) was calculated using the subsequent formula.

$$TD = \frac{Mass\ of\ sample\ in\ g}{tapped\ volume\ occupied\ by\ sample\ in\ ml}$$

Compressibility Index (CI)

The following formula was used to calculate the CI [18].

$$CI = \frac{TD - BD}{TD} \times 100$$

Hausner's Ratio

The following formula was used to calculate Hausner's Ratio (HR) [18].

$$HR = \frac{TD}{BD}$$

Drug content

The spherical agglomerates were triturated using a mortar and pestle. Weighing and dissolving the triturated powder, which equated to 50 mg of ATZR, in 50 ml of 6.8 phosphate buffer required constant stirring for 30 minutes. The drug content was measured at 250.5 nm after filtering the solution using Whatman filter paper No. 41, and the needed ethanol dilutions were prepared [19].

Saturation solubility

Spherical agglomerates from each batch, pure ATZR, were dissolved separately in double-distilled water to make a saturated solution. All solutions were packed in screw-capped glass bottles and kept in an orbital shaker for 3 days. The solutions were centrifuged at 5000 rpm for 30 minutes. After passing the resulting solutions through Whatman filter paper no.41, double-distilled water was used to create the proper dilutions. The solubility of each sample was determined after being analyzed at 250.5 nm using a UV spectrophotometer [20].

FTIR analysis

The FTIR technique was used to obtain the infrared spectra of pure ATZR and optimized spherical agglomerates. The procedure involved preparing potassium bromide pellets. The dry ingredients for each sample were combined in a 1:99 ratio

with potassium bromide, triturated, and then compressed into pellets. The resultant pellets were scanned in the 4000-400 cm-1 frequency band to obtain the spectra.

DSC studies

Pure ATZR and optimized spherical agglomerates were analyzed. A small amount (2 to 3 mg) of ATZR and triturated spherical agglomerates were accurately weighed and placed in separate aluminium pans. The pans were hermetically sealed using a crimper. The DSC analyzer was then filled with the sample and reference pan. At a rate of 100°C per minute, the sample's temperature was raised from room temperature to 400°C. A 100 ml/min flow of nitrogen gas was purged to provide an inert environment.

X-ray diffraction analysis

XRD patterns of optimized spherical agglomerates and pure ATZR were obtained using an X-ray diffractometer fitted with a copper target. The voltage and current used to operate the instrument were 30 kV and 30 mA, respectively. The scanning speed was set at 2°C/min, and the scanning angle ranged from 0 to 90° (2θ).

Residual solvent determination

The residual solvent (methanol and Benzene) from spherical agglomerates was determined using the GC method. 200 mg of spherical agglomerates were weighed accurately and transferred into a headspace vial, and 2 ml of diluent N-methyl-2 Pyrrolidone (NMP) was added and sealed properly. The test sample, the composite std solution, was injected into the GC system (Shimadzu, GC2010 plus with Headspace sampler HS-20) equipped with DB-624 (60m x 0.53mm, 3.0µm) capillary column and flame ionization detector. Nitrogen was used as a carrier gas during analysis. The column flow rate was maintained at 4.59 ml/min. The column oven temperature was maintained between 40 and 230°C, while the detector temperature was set at 240°C. The GC cycle time was 38 min. The residual solvent was determined using the following formula.

$$Solvnet\ (ppm) = \frac{Ru}{Rs} X\ \frac{Ws\ X\ PF}{50} X \frac{5}{100}\ X\ \frac{2}{Wt}\ X1000000$$

Ru: Avg area of individual solvent peak obtained from test chromatogram; Rs: Avg area of respective solvent peak obtained from composite std solution; Ws: Wt of respective solvent in mg Wt: Wt of the test sample in mg; PF: Purity factor

$$Calculation\ of\ purity\ factor\ = \frac{Purity\ of\ respective\ solvent}{100}$$

Formulation and development of ATZR immediate-release (IR) capsules

ATZR capsules contain spherical agglomerates, ATZR capsules contain pure API using the wet granulation method, and ATZR capsules contain pure API using the DC method. These three approaches were used to manufacture the IR capsules. The details of all manufacturing methods are presented below.

Formulation of ATZR capsules containing spherical agglomerates

ATZR IR capsules containing spherical agglomerates were manufactured using the dry granulation method. ATZR agglomerates, Crospovidone, and Lactose monohydrate were co-sifted through #30 and mixed for approximately 20 minutes in a polybag. Magnesium stearate was sifted through #60 and mixed well in a polybag with the above pre-lubricated blend for approximately 5 min. The lubricated blend was filled manually in Size '1' White opaque HGC capsules. The formula composition is presented in Table 3. The lubricated blend and capsules were evaluated further.

Table 3: Formula composition of ATZR IR capsules with spherical agglomerates

ATZR capsules (with spherical granules) (formulation F1)			
S No.	**Ingredients**	**mg/ capsule**	**%**
Dry Mix			
1	ATZR Agglomerates	341.7	89.92
2	Lactose Monohydrate (Pharmatose 200 M)	25.3	6.66
3	Crospovidone (Kollidon CLF)	10	2.63
Lubrication			
5	Magnesium stearate	3	0.79
Total		380	
6	EHG Hard gelatin capsules size "1"	78	

Formulation of ATZR capsules using wet granulation method:

ATZR and Lactose monohydrate were co-sifted through #30 and mixed approximately for 10 min in a polybag. Granulation was performed by hand in SS bowl with purified water. At 50°C, the moist granules were dried in a tray drier at 55°C. To get granules of a consistent size, the dried granules were sieved through # 20.

Crospovidone was sifted through #30 and mixed with dried granules in a polybag for approximately 10 min. Magnesium stearate was sifted through #60 and mixed well in a polybag with the above pre-lubricated blend for approximately 5 min. The lubricated blend was filled manually in Size '0EL0' White opaque HGC capsules. The formula composition is presented in Table 4. The lubricated blend & capsules were evaluated further.

Table 4: Formula composition of DVRE tablets using wet granulation method

ATZR capsules (Wet Granulation) (Formulation F2)			
SNo	**Ingredients**	**mg/tab**	**%**
Dry Mix			
1	ATZR	341.7	63.28
2	Lactose Monohydrate (Pharmatose 200 M)	164.3	30.43
Granulation			
3	Purified Water	qs	qs
Pre-Lubrication			
4	Crospovidone (Kollidon CLF)	29	5.37
Lubrication			
5	Magnesium stearate	5	0.93
Total		**540**	100
6	EHG Hard Gelatin Capsules Size "0EL"	107	

Formulation of ATZR capsules by direct compression method

Pure ATZR, Crospovidone, and Lactose monohydrate were co-sifted through #30 and mixed for approximately 20 minutes in a polybag. Magnesium stearate was sifted through #60 and mixed well in a polybag with the above pre-lubricated blend for approximately 5 minutes. The lubricated blend was filled manually in size '0EL0' white opaque HGC. The formula composition is presented in Table 5. The lubricated blend and compressed tablets were evaluated further.

Characterisation of lubricated blend of F1, F2 & F3 batches

The lubricated blend of F1, F2, and F3 batches was evaluated for micromeritics properties. BD, TD, CI, and HR were determined using the procedure mentioned in the previous section.

Evaluation of Capsules

Weight variation

According to IP guidelines, twenty capsules were randomly selected from each batch. The capsules were weighed using a digital balance, and the average weight was calculated [21]. To determine the weight variation, the individual capsule's weights were compared to the calculated average weight

Table 5: Formula composition of ATZR capsules using a direct compression method

ATZR capsules (dry granulation) (Formulation F3)			
SNo	**Ingredients**	**mg/cap**	**%**
Dry Mix			
1	ATZR	341.7	69.31
2	Microcrystalline Cellulose (GR 102)	121	24.54
3	Crospovidone (Kollidon CLF)	25	5.07
Lubrication			
5	Magnesium stearate	5.3	1.08
Total		**493**	100.0
6	EHG Hard Gelatin Capsules Size "0EL"	**107**	

Capsule lock length

The capsules were filled and locked manually by pressing the cap over the body. The capsule lock length was determined using a vernier caliper.

Content uniformity

To determine the drug content uniformity of the ATZR capsule, the powdered blend of 10 capsules from each formulation was mixed properly. 300 mg of ATZR powder was dissolved in ethanol. After filtering the mixture via filter paper, the absorbance at 250.5 nm was measured using a UV-visible spectrophotometer to ascertain the amount of ATZR present. Dilution was done as needed to guarantee precise readings.

Capsule net content

A representative sample of capsules (n=10) from the batch was collected randomly. All 10 capsules were weighed together to give gross weight (capsule shell weight + content weight). The weight of 10 empty capsules was determined and subtracted from the gross weight to give the net weight of the filled content.

Disintegration time

The DT was performed on six capsules using a disintegration test apparatus. The disintegration medium was 900 ml of distilled water at 37 ± 0.5°C.

Dissolution study

Using 1000 ml of 0.025 N HCl as the release medium and keeping it at 37±0.10C, in vitro dissolution experiments were

carried out in a USP class II dissolution test device. The paddle's speed was set at 50 revolutions per minute. To keep the sink condition, the 5 ml samples were removed at predefined intervals and an equivalent volume of new buffer was supplied. At 250.5 nm, the solutions were analyzed to ascertain the drug release.

RESULTS AND DISCUSSION

Development of ATZR spherical crystals using a bridging solvent

The spherical agglomerates of ATZR were developed using HPMC 5 cps polymer, with methanol as a good solvent. Benzene was used as a bridging solvent, while water acted as a bad solvent.

The good (e.g., Methanol) solvent is where the drug dissolves easily and forms small, well-defined crystals. It dissolves the drug, forming uniform nuclei as starting points for spherical crystal growth. The bad or antisolvent (e.g., Water) is one in which the drug has poor solubility. Adding to the good solvent reduces drug solubility, causing controlled precipitation of small drug particles that act as seed crystals for spherical growth [22]. The bridging solvent (e.g., Benzene) connects drug particles formed in the bad solvent. It has some solubility for both drug particles and growing crystals, facilitating the agglomeration of drug particles around seed crystals and promoting the formation of spherical shapes. Polymers act as templating agents, interacting with solute particles and influencing their nucleation and growth, leading to spherical crystal formation [23].

Evaluation of spherical agglomerates

Flow properties of the spherical agglomerates

The BD of the pure ATZR was found to be 0.406± 0.015 gm/cm3, and TD was found to be 0.542± 0.017. The HR of 1.33± 0.010was observed while the CI was 25.00± 0.015. The spherical agglomerates of ATZR improved the micromeritics properties of pure ATZR as shown in Table 6. The spherical agglomerates' BD, TD, CI, and HR were increased compared to pure ATZR. The BD was ranged between 0.502± 0.010 to 0.516± 0.016 gm/cm3. The TD ranged between 0.595± 0.013 to 0.630± 0.013gm/cm3. HR was found in the range of 1.19± 0.011 to 1.23± 0.010, and CI was found between 15.63± 0.010 to 25.00± 0.015. Overall, the flow properties in all batches were promising [24].

Table 6: Flow properties of the spherical agglomerates and pure ATZR

Sr. No	Formulation	BD	TD	CI	HR
1	**Pure ATZR**	0.406± 0.015	0.542± 0.017	25.00± 0.015	1.33± 0.010
2	**SAA1**	0.508± 0.011	0.595± 0.013	15.63± 0.010	1.19± 0.012
3	**SAA2**	0.510± 0.011	0.623± 0.016	18.14± 0.011	1.22± 0.012
4	**SAA3**	0.509± 0.012	0.611± 0.020	16.69± 0.017	1.20± 0.015
5	**SAA4**	0.502± 0.010	0.605± 0.021	17.02± 0.011	1.21± 0.012
6	**SAA5**	0.516± 0.016	0.623± 0.010	17.17± 0.010	1.21± 0.015
7	**SAA6**	0.515± 0.011	0.630± 0.013	18.25± 0.021	1.22± 0.017
8	**SAA7**	0.504± 0.012	0.598± 0.011	15.72± 0.022	1.19± 0.011
9	**SAA8**	0.503± 0.014	0.601± 0.021	16.47± 0.013	1.20± 0.010

Spherical agglomerates are conglomerates of fine particles agglomerated or bound together into spherical shapes. This process can significantly improve the flow properties of materials, especially in industries like pharmaceuticals and powders [25]. The study conducted by Shivakumar et al. also observed a similar improvement in the flow properties of the spherical agglomerates of Etoricoxib [26]. Spherical agglomerates, formed by binding fine particles into spherical shapes, offer several advantages for materials' flow properties. Their minimal surface contact reduces interparticle friction, enhancing material flowability. These agglomerates are designed for uniform particle size distribution, reducing segregation risks during handling and transport. Spherical particles pack more efficiently, reducing void spaces and optimizing storage. Their uniform shape and size enhance flowability, reducing interruptions and blockages in manufacturing processes. Moreover, they are less likely to form arches or bridges, ensuring continuous material flow. Spherical agglomerates disperse more consistently, are easier to handle, resist caking, and enable precise dosing in pharmaceutical applications. These improve flow properties and overall material handling efficiency [26].

Effect of independent variables on drug content (Y1) and statistical analysis

ATZR spherical agglomerates were developed by combining HPMC, methanol, benzene, and water and optimized using a 2^2-factorial design approach. The formulation batches suggested by design expert software were manufactured and evaluated for drug content, which was considered an independent variable. The drug content results and all the formulations are presented in Table 7, along with the coded levels of the dependent variables.

Table 7: Results of drug content with levels of independent variables

Batch	Factor		Drug content (%)	Solubility (mg/ml)
	A (HPMC)	B (Benzene)	Y1	Y2
SAA1	-1	-1	93.3	10.44
SAA2	+1	-1	97	40.5
SAA3	-1	-1	93.6	11.11
SAA4	-1	+1	91.95	4.88
SAA5	+1	+1	95.6	19.33
SAA6	-1	+1	91.9	4.44
SAA7	+1	+1	95.69	19.99
SAA8	+1	-1	96.8	39.89

The drug content of the spherical agglomerates ranged between 91.9% (SAA6) to 97 % (SAA2). SAA2 agglomerates showed highest drug content than other formulations. The study reveals a clear relationship between drug content and the concentrations of HPMC and benzene in the formulation. Specifically, there is a direct correlation between drug content and higher levels (+1) of HPMC concentration, while an inverse relationship is observed with lower levels (-1) of benzene concentration. Batches formulated with higher HPMC concentrations and lower benzene concentrations consistently exhibit higher drug content. This relationship and the impact of the independent variables on drug content are graphically represented in Figure 1.

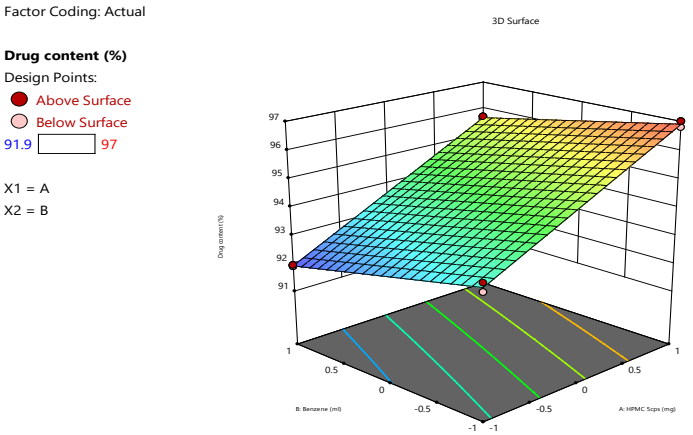

Figure 1: 3D surface responses of HPMC and Benzene on drug content

Increased polymeric concentration plays a pivotal role in enhancing drug encapsulation and binding, leading to higher drug content. In the context of spherical crystallization, the bridging liquid, benzene, serves as a binding agent facilitating the formation of spherical agglomerates from individual drug particles. Our experiments have demonstrated that lower concentrations of this bridging liquid can significantly improve drug content in the spherical crystallization process. With lower concentrations of the bridging liquid, drug particles have a higher likelihood of direct contact with one another. This increased contact promotes the formation of larger drug agglomerates, thereby increasing drug content within the resulting granules or crystals. Additionally, the reduced volume of bridging liquid introduced into the system under lower concentrations minimizes dilution effects on the drug particles, enabling a higher drug concentration within the agglomerates. Furthermore, during the spherical crystallization process, there is a risk of drug dissolution in the bridging liquid, potentially leading to a loss of drug content. However, when the concentration of the bridging liquid is lowered, the available liquid volume for drug dissolution is reduced. This, in turn, mitigates drug loss and contributes to an overall improvement in drug content in the final product [27].

The final polynomial equation for drug content (Y1) in coded factors can be presented below

$$(Y1) = +94.48 + 1.79A - 0.6950B + 0.0675AB$$

In the above equation, Y1 is drug content, A is HPMC concentration, and B is benzene concentration. Model terms are considered significant when P-values are less than 0.0500. A and B are important model variables (Table 8). Table 8 indicates that

the model is significant based on its F-value of 561.50. This huge F-value has a 0.01% probability of being caused by noise. There is less than 0.2 difference between the adjusted R^2 of 0.9976 and the expected R^2 of 0.9905, indicating a fair agreement (Table 8).

Effect of independent variables on solubility (Y2) and statistical analysis

Table 9 presents the solubility results of all the formulations and the coded levels of the dependent variables.

Table 8: ANOVA for selected factorial model of drug content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	29.61	3	9.87	561.50	< 0.0001	significant
A-HPMC 5cps	25.70	1	25.70	1462.56	< 0.0001	
B-Benzene	3.86	1	3.86	219.87	0.0001	
AB	0.0364	1	0.0364	2.07	0.2233	
Pure Error	0.0703	4	0.0176			
Cor Total	29.68	7				
Fit statistics						
Std. Dev.	0.1326		R^2	0.9976		
Mean	94.48		Adjusted R^2	0.9959		
C.V. %	0.1403		Predicted R^2	0.9905		
			Adeq Precision	53.0714		

Table 9: Solubility study results with levels of independent variables

Batch	Factor		Solubility (mg/ml)
	A (HPMC)	B (Benzene)	Y2
SAA1	-1	-1	10.44
SAA2	+1	-1	40.5
SAA3	-1	-1	11.11
SAA4	-1	+1	4.88
SAA5	+1	+1	19.33
SAA6	-1	+1	4.44
SAA7	+1	+1	19.99
SAA8	+1	-1	39.89
Pure ATZR			4.00

The solubility of the spherical agglomerates varied within the range of 4.44 mg/ml (SAA6) to 40.5 mg/ml (SAA2), while the solubility of pure ATZR stood at 4 mg/ml. Remarkably, a significant improvement in solubility was evident when ATZR was subjected to the spherical agglomeration process. Notably, the SAA2 batch exhibited the highest solubility at 40.5 mg/ml, marking a remarkable 10.12-fold increase in solubility compared to the pure ATZR. In a parallel manner to the findings on drug content, our investigations unveiled a negative correlation between solubility and the concentration of the benzene. Conversely, there was a direct positive correlation between solubility and the concentration of HPMC. Specifically, the solubility of spherical agglomerates increased when formulated with higher levels (+1) of HPMC and lower levels (-1) of benzene. These relationships and the influence of the independent variables on solubility are visually depicted in Figure 2.

Increased polymeric content in spherical crystallization enhances drug solubility through several mechanisms. The higher concentration of polymers promotes improved wetting of drug particles, facilitates strong interactions between drug and polymer, increases surface area through solid dispersion formation, stabilizes the amorphous state, and reduces particle

size. These polymers effectively reduce interfacial tension, enhance dissolution, and prevent particle aggregation. They disperse drug molecules within the polymer matrix, enhance drug-polymer interactions, and inhibit recrystallization. Additionally, polymers aid in reducing particle size, preventing crystal growth, and promoting the formation of smaller, more uniformly sized drug particles [28].

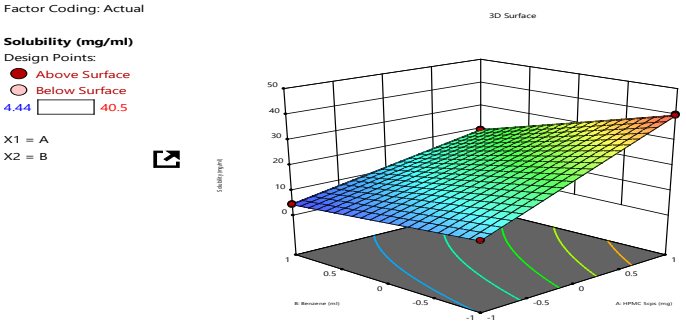

Figure 2: 3D surface responses of HPMC and benzene on solubility

Higher polymeric content enhances drug solubility in spherical crystallization through many mechanisms. Conversely, lower concentrations of the bridging solvent in spherical crystallization also contribute to improved drug solubility. This is achieved through mechanisms such as supersaturation and precipitation, resulting in the formation of drug crystals. Lower solvent concentrations lead to particle size reduction, thereby increasing the surface area available for dissolution. Controlled crystal growth at lower concentrations allows for the creation of more soluble crystal forms. Additionally, reduced solvent concentration influences crystal morphology, favoring the development of desirable spherical shapes that enhance flow properties and dissolution [27]. Overall, lower bridging solvent concentration promotes supersaturation, particle size reduction, controlled crystal growth, and morphology control, all contributing to enhanced drug solubility in spherical crystallization.

The final polynomial equation for drug content (Y1) in coded factors can be presented below.

$$Y2 = +18.82+11.11A-6.66B-3.61AB$$

In the above equation, Y2 is drug solubility, A is HPMC concentration, and B is benzene concentration. Model terms are considered significant when P-values are less than 0.0500. A, B, and AB are important model terms (Table 10). Table 10 indicates that the model is significant based on its F-value of 2658.30. This huge F-value has a 0.01% probability of being caused by noise. The corrected R^2 of 0.9985 and the expected R^2 of 0.9966 are reasonably in agreement; the difference is less than 0.2 (table 10).

Table 10: ANOVA for selected factorial model of drug solubility

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	1445.65	3	481.88	2658.30	< 0.0001	significant
A-Plasdone 630	986.57	1	986.57	5442.38	< 0.0001	
B-MDC	355.11	1	355.11	1958.96	< 0.0001	
AB	103.97	1	103.97	573.54	< 0.0001	
Pure Error	0.7251	4	0.1813			
Cor Total	1446.37	7				
Fit statistics						
Std. Dev.	0.4258		R^2	0.9995		
Mean	18.82		Adjusted R^2	0.9991		
C.V. %	2.26		Predicted R^2	0.9980		
			Adeq Precision	118.0327		

FTIR analysis

The distinct peaks in ATZR's FTIR spectrum correlate to the various functional groups that the molecule contains (Figure 3A). The spectrum typically consisted of strong peaks in the 3100-3000 cm^{-1} region, which are attributed to C-H stretching vibrations in the aromatic rings of Atazanavir. In the 3000-2850 cm^{-1} region, additional peaks correspond to C-H stretching vibrations in aliphatic (non-aromatic) parts of the molecule. Around 3300-3500 cm^{-1}, a broad and strong peak is associated with N-H stretching vibrations. This peak is typically attributed to the amide group in ATZR. The amide group also contributed to 1650-1680 cm^{-1} peaks, corresponding to C=O stretching

vibrations. This region is often referred to as the amide I band. The 1200-1300 cm^{-1} characteristic peak is present due to C-N stretching vibrations within the amide group. This region is known as the amide III band. Ether or hydroxyl groups in the molecule are observed in the 1000-1300 cm^{-1} range, attributed to C-O stretching vibrations. The region below 1500 cm^{-1}, often referred to as the fingerprint region, contains various smaller peaks unique to the molecular structure of ATZR. Similar characteristic peaks were also observed at the same wavenumber with reduced intensity in ATZR-loaded spherical agglomerates (Figure 3B). This observation indicated the excellent compatibility of the drug with the polymer used in the formulation.

XRD analysis

The presence of well-defined and sharp peaks in the diffractogram confirmed the crystalline nature of ATZR (Figure 4A). However, in the XRD pattern of ATZR-loaded spherical agglomerates, we observed broad peaks and diffuse scattering, indicating a lack of long-range order and the presence of a disordered or amorphous structure. Notably, the XRD spectra of ATZR-loaded spherical agglomerates displayed smaller and less pronounced peaks when compared to the pure ATZR (Figure 4B). This observation strongly suggests that the drug underwent amorphization during the agglomeration process.

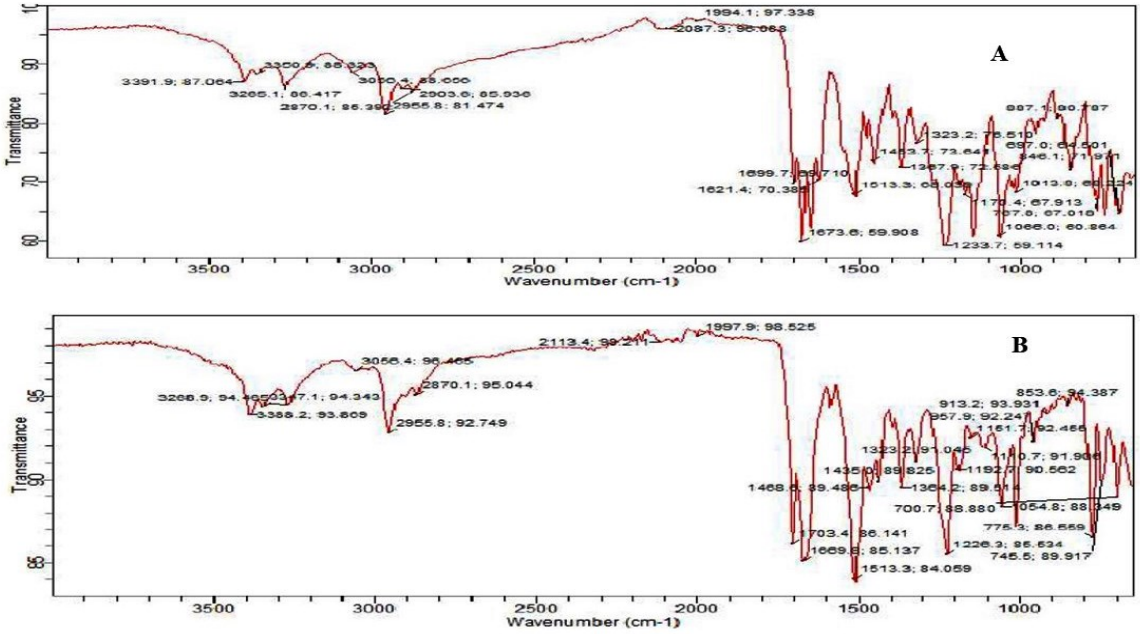

Figure 3: FTIR spectra of A: pure ATZR and B: ATZR loaded spherical agglomerates

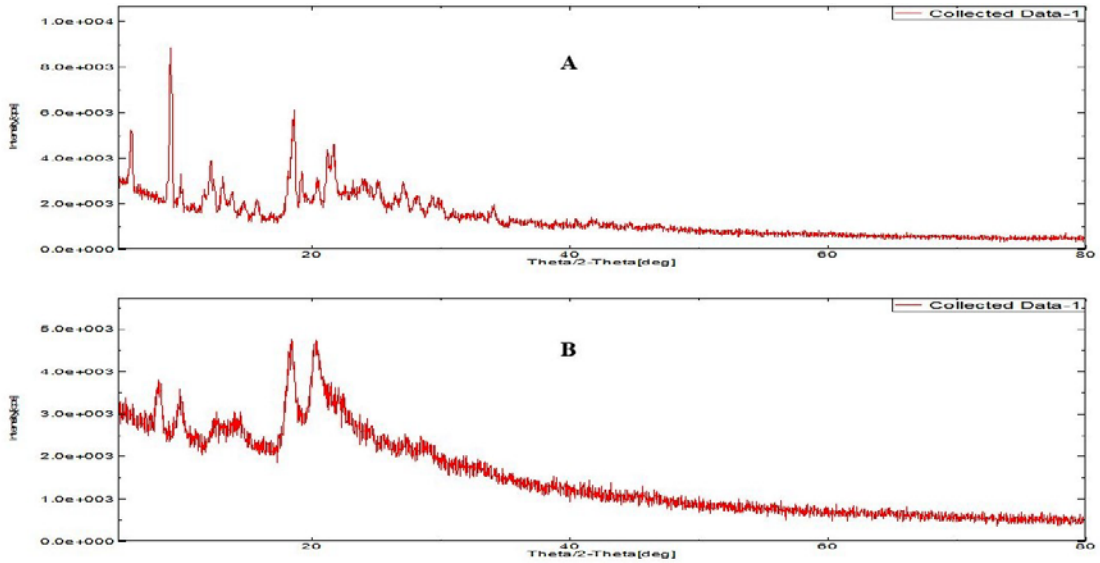

Figure 4: X-ray diffractogram of A: Pure DVRE; B: DVRE loaded spherical agglomerates

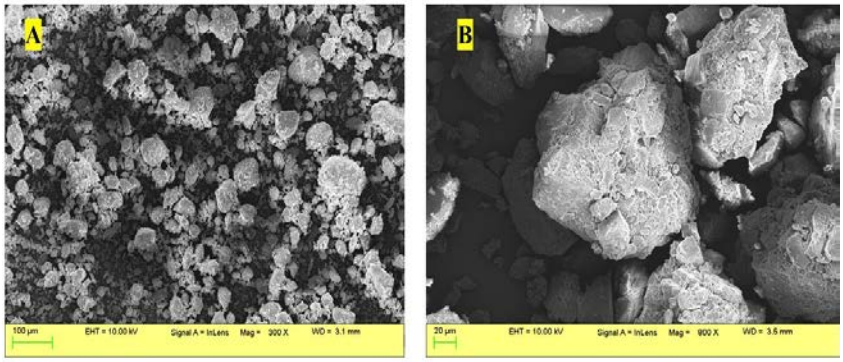

Figure 5: Scanning electron microscopy (SEM) of A: Pure ATZR; B: ATZR loaded spherical agglomerates

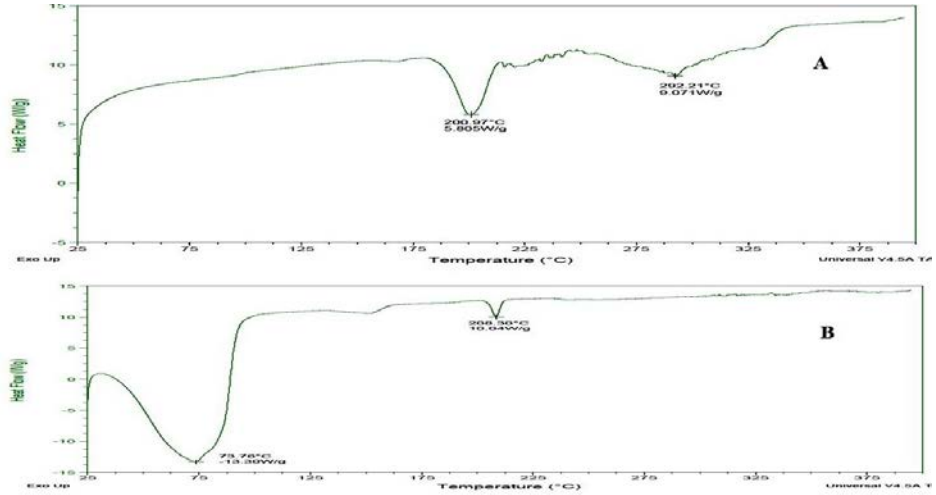

Figure 6: DSC thermograph of A: Pure ATZR, B: ATZR loaded spherical agglomerates

Scanning electron microscopy (SEM)

The SEM images indicated the irregular structure of the pure ATZR with rough surface properties. The particles were also found to be larger (Figure 5A). The ATZR-loaded spherical agglomerates, on the other hand, had the proper spherical shape with smooth surfaces, as observed in Figure 5B.

DSC analysis

The DSC analysis revealed a sharp endothermic peak of pure ATZR at 208.30°C, corresponding to its melting point ranging from 195 to 209°C, indicating its crystalline nature (Figure 6A). In contrast, ATZR-loaded spherical agglomerates showed an endothermic peak at 200.97°C. This slight shifting of the peak indicated the amorphization of the drug within the polymeric matrix (Figure 6B). This disappearance of the endothermic peak at 208.30°could be attributed to the encapsulation of ATZR within the polymeric matrix.

Residual solvent determination

While manufacturing ATZR-loaded spherical agglomerates, methanol, and benzene were used as solvents. The residual amount of these solvents present in spherical agglomerates was determined to check whether the agglomerates meet the regulatory requirement of residual solvent criteria. The residual methanol content in agglomerates was 400 ppm, which falls within the acceptance criteria of 3000 ppm. For benzene, the maximum allowed residual concertation is 2 ppm, and in spherical agglomerates, benzene concentration was found to be not detectable, which is also within the acceptance limit [24]. These observations demonstrate that the ATZR-loaded spherical agglomerates meet the residual solvent criteria for methanol and benzene.

Formulation and development of ATZR IR capsules

Characterization of lubricated blend

The lubricated blend of F1, F2, and F3 batches was evaluated for micromeritics properties, including BD, TD, CI, and HR. The comparative outcome from all batches is presented in Table 11. The lubricated blend manufactured in the F1 batch showed higher BD and better flow properties than those manufactured in the F2 and F3 batches. The compressibility index in the case of F2 (33.84) and F3 (36.36) was found to be very poor (32-37

considered as very poor), while the F1 blend (18.51) showed good compressibility (11-15 considered as fair). Similarly, HR was also very poor (1.46 to 1.59) in the case of F2 and F3 blends. F1 lubricated blend showed good HR (1.11 to 1.18). These observations indicated that the use of ATZR spherical agglomerates has significantly improved the flow properties and compressibility of the blend compared to pure DVRE. Spherical agglomerates of drugs offer several advantages in pharmaceutical manufacturing, primarily by enhancing flow properties and compressibility. They reduce cohesion between particles due to their smooth and uniform surface, improving flowability. The lower angle of repose ensures consistent and efficient filling during capsule or tablet compression. Additionally, they minimize the risk of particle segregation during handling. Regarding compressibility, spherical agglomerates enable greater packing density, resulting in higher bulk density and better tablet compressibility. They also reduce the potential for air entrapment during tablet compression, preventing defects and promoting a more even distribution of lubricants and excipients in tablet formulations, ultimately enhancing tablet quality and uniformity [29]. These attributes make spherical agglomerates valuable in pharmaceutical production.

Table 11: Comparative micromeritics properties of the lubricated blend

Formulation	BD	TD	CI	HR
F1 (DC blend with Spherical agglomerates)	0.611	0.750	18.51	1.22
F2 (Wet Granulation blend with pure ATZR)	0.415	0.628	33.84	1.51
F3 (DC blend with pure ATZR)	0.333	0.524	36.36	1.57

Evaluation of ATZR capsules

Table 14 presents the comparative evaluation parameters of the capsules. The comparative evaluation parameters of the ATZR capsules showed that the F1 formulation, manufactured with a DC blend of Spherical agglomerates, showed promising results with minimum DT and higher drug content compared to the rest of the formulations, as shown in Table 12.

Table 12: ATZR capsule evaluation parameters

S No	Formulation	Weight	Capsule net content (%)	Lock length (mm)	DT (min)	% yield
1	F1 (DC blend with Spherical agglomerates)	458±3%	380±3%	19.5±0.3	7-8	98.00
2	F2 (Wet Granulation blend with pure ATZR)	647±3%	540±3%	23.5 ± 0.3	10-15	95.12
3	F3 (DC blend with pure ATZR)	600±3%	493±3%	23.5 ±0.3	10-12	98.20

According to the USP for capsules, all the batches comply with the weight variation test. According to USP, capsules weighing more than 300 mg have a weight variation limit of 7.5%. In all batches, the weight variation was found within acceptable limits [30]. F2 and F3 batches showed DT of 10-15 min and 10-12 min, respectively, while the F1 batch showed DT of 7-8 min. The presence of spherical agglomerates might have helped with the rapid disintegration of the capsules in comparison to pure ATZR in other batches. Also, a higher drug content of 98.00% was found in the F1 formulation, possibly due to the uniform mixing of the spherical agglomerates with other excipients of the capsules.

Dissolution study

A dissolution study was performed in 0.025 N HCl. The comparative release profile of all formulations is presented in Figure 7. Nearly 100% release was observed within 10 minutes from the F1 formulation containing ATZR spherical agglomerates. Complete drug release was not observed even in a time period of 1 hour from F2 (88%) and F3 (69%) formulations.

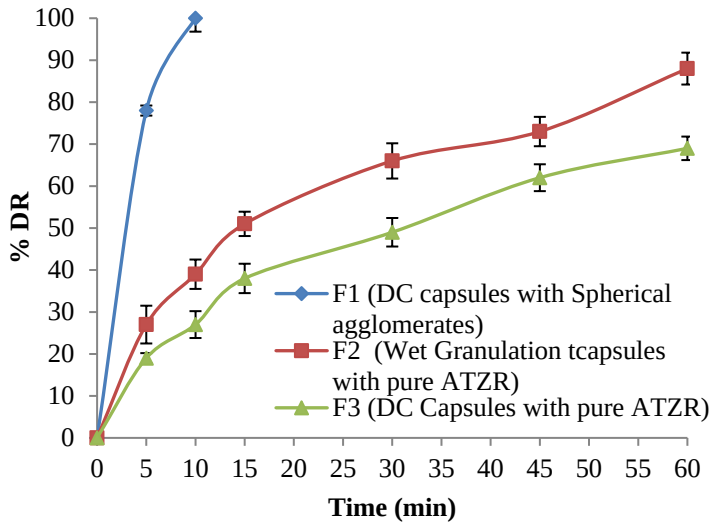

Figure 7: Comparative ATZR release from IR capsules

Spherical agglomerates of drugs offer several advantages for drug release enhancement. They possess a larger surface area, uniform particle size distribution, and improved wettability, facilitating efficient contact with the dissolution medium, reducing the risk of agglomeration and clumping, and promoting consistent and predictable drug release. Their superior flow properties aid in uniform tablet manufacturing, contributing to consistent drug distribution within tablets. Reduced interparticle cohesion further ensures smoother particle separation during dissolution. Additionally, pharmaceutical companies can tailor release profiles by adjusting agglomerate properties. Finally, their enhanced compressibility makes them suitable for tablet production, ensuring tablet structural integrity and contributing to reliable drug release.

CONCLUSION

This study successfully developed spherical crystals of ATZR using a blend of HPMC, methanol, water, and benzene, demonstrating substantial improvements in this high-dose molecule's flow properties, solubility, and dissolution rate. The spherical agglomeration technique enhanced micromeritic properties, including BD, TD, HR, and CI, resulting in superior flow characteristics compared to ATZR in its unprocessed form. These advancements are crucial for improving drug formulation, therapeutic efficacy, and patient compliance. However, the study is limited by its focus on laboratory-scale processes. Future research should focus on scaling up the spherical agglomeration process, optimizing solvent recovery and batch size, conducting long-term stability studies, and exploring safer alternatives. These efforts will ensure industrial feasibility, regulatory compliance, and enhanced stability for broader pharmaceutical applications. In today's context, where optimizing drug delivery is vital for addressing complex diseases and improving patient outcomes, these findings hold significant promise for enhancing pharmaceutical formulations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Sanjay S. Pekamwar designed the study and planned the work with its aim and objectives, reviewed the manuscript, and edited the article. Ashok T. Jadhav performed the work, collected data, and wrote the manuscript

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