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PhD Thesis

APPLICATION OF HIGH-SHEAR GRANULATOR IN DIFFERENT PELLETIZATION TECHNIQUES

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PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. **Azza A. K. Mahmoud**, Alharith A. A. Hassan, Dorina Gabriella Dobó, Krisztina Ludasi, László Janovák, Géza Regdon Jr., Ildikó Csóka, Katalin Kristó: Investigation of the Electrokinetic Potential of Granules and Optimization of the Pelletization Method Using the Quality by Design Approach, Pharmaceutics **2024**, 16, 848, DOI 10.3390/pharmaceutics16070848

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II. **Azza A. K. Mahmoud,** Géza Regdon Jr., Katalin Kristó: Cutting-Edge Approaches in the Co-Amorphization Process, Pharmaceutics **2025**, 17, 850, DOI 3390/pharmaceutics17070850

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III. **Azza A. K. Mahmoud**, Krisztina Ludasi, Dorina Gabriella Dobó, Dániel Sebők, Ákos Kukovecz, Viktória Hornok, Kadosa Sajdik, Tamás Szabó, Tamás Sovány, Géza Regdon Jr., Katalin Kristó: The development and characterization of layered pellets containing a combination of amorphized amlodipine besylate and hydrochlorothiazide using high-shear granulator, Pharmaceuticals **2025**, 18, 1496, DOI 10.3390/ph18101496

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I. Yusra Ahmed, **Azza A K Mahmoud**, Krisztina Ludasi, Tamás Sovány: Advances in Loading Techniques and Quality by Design for Fused Deposition Modeling in Pharmaceutical Production: A Systematic Review, Pharmaceuticals **2024**, 17, 1496, DOI 10.3390/ph17111496

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II. **Azza A.K. Mahmoud**, Géza Regdon jr., Katalin Kristó: Determination of design space for direct pelletization using Pro-Cept granulater, *V. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Hungary,* ***January 2023***

III. **Azza A. K. Mahmoud,** Géza Regdon jr., Katalin Kristó: Application of Pro-CepT granulator for direct pelletization process, Young Technologists Forum, Budapest, Hungary, ***May 2023***

IV. **Azza A. K. Mahmoud,** Géza Regdon jr., Ildikó Csóka, Katalin Kristó: High shear granulator as promising technique in direct pelletization process, *VI. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Hungary,* ***January 2024***

V. **Azza A. K. Mahmoud,** Yusra Ahmed, Géza Regdon jr., Katalin Kristó: Combined risk assessment and principal component analysis as effective tools in direct pelletization process, *Congressus Pharmaceuticus Hungaricus XVII. and EUFEPS Annual Meeting, Debrecen, Hungary,* ***May 2024***

VI. **Azza A. K. Mahmoud,** Orsolya Jójárt-Laczkovich, Géza Regdon jr., Katalin Kristó: Preparation of layered pellets loaded with co-amorphous system, *VII. Young Technologists' Forum, Budapest, Hungary,* ***November 2024***

VII. **Azza A. K. Mahmoud,** Dániel Sebők, Géza Regdon jr., Katalin Kristó: Amorphization of Amlodipine Besylate and Hydrochlorothiazide in High Shear Granulator, *VII. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Hungary,* ***January 2025***

VIII. **Azza A. K. Mahmoud**, Dániel Sebők, Ákos Kukovecz, Géza Regdon jr., Katalin Kristó: *Drug amorphization using layering pelletization, VIII. Young Technologists Forum, Budapest, Hungary,* ***May 2025***

IX. **Azza A. K. Mahmoud**, Dániel Sebők, Ákos Kukovecz, Géza Regdon jr., Katalin Kristó: *Pro-Cept granulator as an effective tool for drug amorphization, XXVIII. Spring Wind Conference, Budapest, Hungary,* ***June 2025***

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ABBREVIATIONS

QbD	Quality by Design
DOE	Design of Experiment
API	Active pharmaceutical ingredient
MCC	Microcrystalline cellulose
CMAs	Critical Material Attributes
CPPs	Critical Process Parameters
CQAs	Critical Quality Attributes
QTPPs	Quality Target Product Profiles
FMEA	Failure Mode and Effect Analysis
D50	Median particle size
BCS	Biopharmaceutical Classification System
DSC	Differential scanning calorimetry
FTIR	Fourier-transform infrared spectroscopy
PVP	Polyvinyl pyrrolidone
XRPD	X-ray powder diffraction
Micro-CT	Micro-computed tomography

1. INTRODUCTION

Solid dosage forms are most commonly used to ensure the administration of a safe drug dose with the required therapeutic effect and good patient compliance. It offers an accurate and uniform dose application with good drug stability. Among the different techniques to manufacture solid dosage forms, pelletization is considered a fundamental technique in the pharmaceutical industries to manufacture tablets and capsules. It produces uniformly distributed spherical particles with improved flow properties, lower toxicity and required drug release behavior, either immediate or controlled release behavior [1–3].

This technique can be performed using different methods such as extrusion spheronization pelletization, layering pelletization, spray congealing, spray drying, compression and cryo-pelletization. Some of those methods like direct pelletization and layering pelletization can be applied by high-shear granulator equipment that produces pellets in one step with minimum time and lower cost [4,5].

This equipment is commonly used to produce a multiparticulate system either granules or pellets, due to uniform mixing of ingredients, high loading of APIs and minimum process time. However, it is contained many parameters like mixing bowl design, chopper speed and impeller speed that affected particle size and distribution beside it is required an accurate optimization that can be obtained by quality by design (QbD) tools, including risk assessment and design of the experiment (DoE) to obtain the required quality and improve process reproducibility [6].

2. LITERATURE BACKGROUND

2.1. Pelletization technique

Pellets are a discrete spherical multiparticulate system in a size range (500- 1500) µm with good flow properties and uniform distribution of active pharmaceutical ingredients (APIs). This system is useful for designing a single dosage form containing different API with a synergistic effect and masking unpleasant taste. Moreover, it contributes to enhancing drug dissolution and consequently drug bioavailability as well as reducing drug toxicity and gastrointestinal tract irritation due to decreased dose dumping in addition to improving tablets and capsules manufacturing through improving flowability, minimizing dust and powder segregation. However, this technology contained a large number of material attributes and process parameters, especially in the scale-up process [1,7].

Extrusion spheronization is the most applied method to produce pellets in multiple steps including the use of a granulating binder to form a wet mass then producing extrudate shapes by using an extruder and converting it to spherical particles inside a spheronizer. Despite this process producing narrowly distributed pellets with optimum size, it has a long processing time, requires well optimization of many factors, and is not suitable for moisture-sensitive drugs. In contrast, hot melt extrusion, spray drying and spray congealing are highly costly, not suitable for thermolabile drugs (hot melt extrusion and spray congealing) due to the melting of the drug and require optimization of process variables (spray drying) [4,5].

2.1.1. Direct pelletization

Applying high-shear mixing enhances blending and binding of dry powder with binder to obtain a multiparticulate system, either pellets or granules [4]. This process depends on a lot of factors involving impeller speed, which exhibited better roundness, reduced particle size and porosity when increased at lower ranges, while increasing it at high ranges led to a decrease in roundness [2,8–10].

The effect of impeller speed is related to other factors, such as increasing the liquid amount with high impeller speed, led to an increase in particle size and modifies the particle size distribution [16]. On the other hand, the chopper speed effect is accompanied with high impeller speed on particle size and it generally decreases the particle size and exhibits uniform distribution [2,3,11], but it did not affect particle porosity and hardness [11,12].

Using a highly viscous binder with high impeller speed had a negative effect on particle size, porosity and drug release [3,13] and this disagrees with Chitu et al., who mentioned that a high viscous binder led to an increase in particle size with lower consolidation [9]. Moreover, binder concentration had a proportional relationship with particle hardness and size [2] while the binder addition method and addition rate did not affect the previous characteristics [8,11] and this disagrees with other research that mentioned the addition rate of binder negatively and non-linearly positively affected particle size [10,14].

2.1.2. Layering pelletization

Loading of liquid or solid form of API on inert carrier (core of spherical particles) such as saccharide or microcrystalline cellulose, or tartaric acid is utilized to enhance drug release profile, as well as masking of the unpleasant taste of the drug [15–17]. Microcrystalline cellulose (MCC) is commonly used as the pellets core due to its uniform spherical surface, good hardness and small friability when compared with saccharide carrier [18,19]

This process, particularly the layering of drug suspension, exhibited better drug loading than powder and solution layering [20]. Liquid layering requires an appropriate estimation of atomization type, atomizer speed, application rate of binder, binder concentration and mechanism of drying. In contrast, loading of powder layering had lower process variables and it is suitable for the preparation of hygroscopic drugs with better surface texture due to the absence of a liquid binder [1,19].

High-shear granulator and fluidized bed granulator produce physical force without applying heat or solvent in one step to perform layering pelletization [21,22] and this may lead to the transfer of crystal drug form to amorphous form and consequently improved dissolution rate of loaded drugs.

2.2. Drug amorphization

The amorphization process is commonly used to enhance the drug release of poorly water-soluble drugs by converting the arranged physical crystalline form to an amorphous form; however, this physical form is thermodynamically unstable and easily recrystallizes upon storage. Many strategies were applied to improve the stability of amorphous form, such as solid dispersion, that based on the utilization of a high amount of polymer with low drug quantity in addition to the miscibility problem. This technique is substituted by co-amorphization, that based on the use of a small amount of low molecular weight co-former, either an excipient or another drug that has a good chemical attraction with the target drug to obtain a homogenous co-amorphous system with high drug release and good physical stability [22]. The production of this amorphous form requires a suitable selection of the process temperature to reduce the ability of recrystallization, in addition to the utilization of an appropriate co-former, which is typically a low molecular weight material such as an amino acid, saccharide, organic acid, other drugs, flavonoid, alkaloid, or bile acid. All of those ingredients can form ionic bonds or hydrogen bonds or a π-π bond, or van der Waals force that can stabilize the produced amorphous drug [23–25]. Among different co-formers, applying the drug co-former exhibited a better dissolution rate and higher therapeutic outcome due to the synergetic effects with lower toxicity of the two drugs [26–28].

Drug amorphization can be carried out by many methods, like ball milling, spray drying, and melt quenching, which produce powder as the final product that requires more production processes, such as granulation and milling, to convert it to final solid dosage forms[22]. Recently, crystal indomethacin was converted to an amorphous form and it was loaded on pellets with MCC core by performing layering pelletization within high-shear granulator; however, it had lower stability that was enhanced by adding L-arginine, which acts as a co-former and it made a strong binding with indomethacin [21,22].

2.3. Application of Quality by Design Approach

Quality by Design (QbD) is utilized to produce a highly qualified pharmaceutical product with an extended lifecycle through a building system that focuses on the process of formulation of the drug step by step starting from pre-formulation studies until validation of the end-product. This approach is based on many principles, including:

1. Quality Target Product Profile (QTPPs): involves the desired quality of the final pharmaceutical product such as efficacy and safety.

2. Critical Quality Attributes (CQAs): involve product characteristics (chemical, physical and biological) that are appropriately selected within specific range to obtain required quality.

3. Critical Material Attributes (CMAs): all material characteristics that must be optimized to formulate dosage form within the required quality.

4. Critical Process Parameters (CPPs): manufacturing factors variables that had effect on product quality [29].

Moreover, performing risk assessment to estimate the risk value of materials attributes and process parameters on product quality using different method like a fishbone diagram, that made a descriptive summarization of all process variables, in addition to failure mode and effect analysis (FMEA) that is considered a qualitative method to calculate risk value by multiplying results of detection, severity and probability [30,31]. Moreover, the risk estimation matrix and Pareto chart are visual qualitative methods that classify variables into low, medium and high risk factors through calculating the severity score that resulted from evaluating the relationship between QTPPs and CQAs beside CQAs and the combination of CMAs with CPPs [32].

After that, applying the design of experiment (DOE) is considered the final step to determine the best process variable ranges (design space) that can lead to producing a desired quality of pharmaceutical product with the lowest experimental runs, less time, and resource consumption [33,34].

According to previous literature, QbD approach has been utilized in many studies that mainly focused on formulation of granules by high-shear granulation including the application of many DoE such as Plackett-Burman and Central Composite Design (CCD) to demonstrate effect of different factors (chopper speed, massing time, impeller speed, drying time and water volume) and estimation of design space of particles size and bulk density [35] in addition to the use of FMEA to determine risk factors, combined with Monte Carlo simulation and multivariate analysis to construct a design space to obtain the required median particle size (D50) and drug release percentage [36]. Also, studying the effect of process temperature related to chopper speed and impeller speed on granule size, breaking hardness, surface free energy, aspect ratio and enzyme activity by applying the three-level full factorial design [37].

Although a lot of research mentioned the use of different QbD tools in the pelletization process, there is insufficient information about the effect of the interaction of process parameters with each other on CQAs of pellets.

2.4. Pellets evaluation

Multiparticulate systems containing drugs are characterized using size estimation to confirm they fulfill the requirement of pellet size range between 500-1500 μm as well as detection if it have a narrow size distribution that is measured for example, by sieving or a microscope. On the other hand, aspect ratio can be measured by an optical microscope [5,38,39].

Moreover, flow properties and density are determined to ensure good manufacturing of tablets and capsules by the solvent-displacement method or an air-comparison pycnometer for true density, whereas the stampfvolumeter (automated tapper) is used for bulk density [5].

The mechanical properties, such as breaking hardness, affect the ability of the pellet to resist the mechanical force during coating is estimated by a texture analyzer [38] while friability is determined by a friabilator or shaking the pellets in a Turbula mixer for a fixed period with aid of glass beads that produce additional mechanical force on pellets [5].

Furthermore, one of the main advantages of manufacturing pellets dosage forms is enhancing the drug release, which can be studied by performing a dissolution test according to the pharmacopeial book [5,40].

2.5. Active pharmaceutical ingredients

A multi-particulate system is one of the commonly delivered systems that is suitable for a large number of drugs, particularly biopharmaceutics system class (BCS) II and IV, as it contributes to enhancing drug dissolution as well as producing different drug release patterns involving immediate, delayed and sustained release behavior based on the applied polymer. Additionally, it can be utilized to mask unpleasant taste that making it suitable for pediatric patients [41].

Many studies were performed to improve dissolution and bioavailability of hydrochlorothiazide (BCS II) by applying different pelletization techniques, such as extrusion-spheronization with polyethylene glycol 200 (liqui-pellet formulation) [42]. Also, polyethylene glycol 400 is used in combination with castor oil as a co-solvent to enhance the dissolution of the hydrochloethiazide pellet [43].

Moreover, it was formulated as a pellets dosage form with ramipril after optimization of factors of extrusion spheronization technique and it exhibited enhanced release behavior [44]. On the other hand, it exhibited a transformation of it crystalline physical form that enhanced dissolution rate in many studies through combining it with many drugs, such as simvastatin [45], atenolol [46] and telmisartan [47] in addition to applying arginine (co-former) [48] to prepare a co-amorphous system and cyclodextrin (solid dispersion) [49].

On the other hand, amlodipine besylate has good water solubility and it forms a co-amorphous preparation with candesartan with enhanced dissolution and stability, especially in polyvinylpyrrolidone-containing formulation [50].

Furthermore, hypertension treatment that contains a combination of hydrochlorothiazide and amlodipine besylate is widely used due to better efficacy compared to single drugs in decreasing blood pressure and reducing the adverse metabolic effects [51].

3. AIM AND OBJECTIVES

This study aims at the preparation of amlodipine besylate and hydrochlorothiazide pellets using direct pelletization and layering pelletization techniques in a high-shear granulator. This aim was divided into the following objectives:

I. Investigating and screening QTPPs, QCAs, CMAs and CPPs that affect the direct pelletization process.

II. Estimation of high-risk factors affecting pellets quality and optimization of the design space suitable for different drug loading.

III. Loading of amlodipine besylate and hydrochlorothiazide in the optimized formulation and performing a comparative evaluation of the resulting pellets.

IV. Investigation of the electrokinetic potential characteristics of the optimized pellets formulation.

V. Determination of the optimum conditions for formulation of layered pellets loaded with a mixture of amlodipine besylate and hydrochlorothiazide.

VI. Investigation of the effect of a high-shear granulator on the amorphization and enhancing release percent of crystalline drugs.

4. MATERIALS AND METHODS

4.1. Chemicals

In this study, hydrochlorothiazide and amlodipine besylate which were obtained from Sigma-Aldrich (St. Louis, MO, USA), were selected as model APIs. Distilled water and polyvinyl pyrrolidone (PVP) (Hungaropharma Zrt, Budapest, Hungary) were utilized as a binder for direct pelletization, whereas talc (Molar Chemicals Kft, Budapest, Hungary) was used as a glidant in layering pelletization.

Moreover, Microcrystalline cellulose (MCC, Vivapur 102) was supplied from JRS Pharma (Patterson, NY, USA), while cellets containing microcrystalline cellulose (Pharmatrans Sanaq Ag, Basel, Switzerland) that were applied as a core for layering pelletization with a particle size of 1000 μm and mannitol were purchased from Hungaropharma Zrt. (Budapest, Hungary).

In the evaluation stage, FTIR potassium bromide for FTIR measurement was supplied from J&K Scientific Limited (Beijing, China) while the phosphate buffer (pH = 6.8) (Ph. Eur.) and hydrochloric acid solution (0.1 N) (Ph. Eur.) that were utilized as media for the dissolution test.

4.2. Direct pelletization

4.2.1. Risk Assessment

According to preformulation and previous literature studies, CQAs and QTPPs were estimated. After that, the Ishikawa diagram were prepared to collect process factors and corresponding required characteristics, whereas the Pareto chart risk and risk estimation matrix (REM) were designed to estimate high-risk factors using LeanQbD software (QbD Works LLC, Fremont, CA, USA/Version 1.3.6, 2014).

4.2.2. Preparation of Pellets

Direct pelletization was carried out by applying of Turbula mixer (Willy A. Bachofen Maschinenfabrik, Basel, Switzerland) for mixing of mannitol (40 g) and MCC (60 g) with PVP at different quantities (1, 1.5 and 3 g) that depend on DoE. After that, the produced mixture was kneaded with distilled water in a Pro-CepT granulator (ProCepT NV, ZelZate, Belgium) then the pellets were dried in an air-ventilated oven (Memmert GmbH+Co. KG, Büchenbach, Germany) at 40 °C for 2 hours. The design space was constructed to obtain the required CQAs then amlodipine besylate and hydrochlorothiazide were loaded in the optimized experiment.

4.2.3. Selection of Design of Experiment (DoE)

The selected high-risk factors and their levels were mentioned in *Table 1*, then three types of design were performed as follows:

Table 1. *Process parameters with selected high, center and low levels*

Factors	Factors name	Low level (−1)	Center level (0)	High level (+1)
X_1	Impeller speed (rpm)	500	1000	1500
X_2	Chopper speed (rpm)	1000	1500	2000
X_3	Liquid volume (mL)	55	57.5	60
X_4	Binder amount (g)	1	2	3
X_5	Addition rate (mL/min)	5	7.5	10

4.2.3.1. Screening Design

According to risk assessment results, 5 variables (impeller speed, chopper speed, granulating liquid amount, binder concentration and liquid addition rate) were included in screening design with 8 experiments that were designed by Statistica software, version 13 (TIBCO Software Inc., Palo Alto, CA, USA) (*Table 2*).

Table 2. *Screening design*

Experiment No.	Impeller speed (rpm)	Chopper speed (rpm)	Liquid addition rate (mL/min)	Granulation liquide (mL)	Binder amount (g)
1	500	1000	10	55	3
2	1500	1000	5	55	1
3	500	2000	5	55	3
4	1500	2000	10	55	1
5	500	1000	10	60	1
6	1500	1000	5	60	3
7	500	2000	5	60	1
8	1500	2000	10	60	3

4.2.3.2. Two-Level Full Factorial Design

16 experiments with one center point were performed according to DoE (*Table 3*). The dependent factors include Impeller speed (X_1), chopper speed (X_2), granulating liquid amount (X_3) and binder amount (X_4), whereas the dependent responses include yield percent, hardness and aspect ratio.

Table 3. *Two-Level Full Factorial Design*

Experiment No.	Impeller speed (rpm)	Chopper speed (rpm)	Granulating liquid volume (mL)	Binder amount (g)
1	500	1000	55	1
2	1500	1000	55	1
3	500	2000	55	1
4	1500	2000	55	1
5	500	1000	55	3
6	1500	1000	55	3
7	500	2000	55	3
8	1500	2000	55	3
9	500	1000	60	1
10	1500	1000	60	1
11	500	2000	60	1
12	1500	2000	60	1
13	500	1000	60	3
14	1500	1000	60	3
15	500	2000	60	3
16	1500	2000	60	3
17(c)	1000	1500	57.5	2

4.2.3.3. Central Composite Design

This design was applied by adding an additional 10 experiments with the same independent and dependent variables of a two-level full factorial design (*Table 4*).

Table 4. *Central composite design*

Experiment No.	Impeller speed (rpm)	Chopper speed (rpm)	Granulating liquid volume (mL)	Binder amount (g)
18	1500	1500	57.5	2
19	1000	1000	57.5	2
20	1000	2000	57.5	2
21	1000	1500	55.0	2
22	1000	1500	60.0	2
23	1000	1500	57.5	1
24	1000	1500	57.5	3
25	1000	1500	57.5	2
26	1000	1500	57.5	2
27	500	1500	57.5	2

4.2.4. Physical properties of pellets

4.2.4.1. Yield Percent and Size Distribution

A series of sieves (Retsch GmbH, Haan, Germany) at size between 500 to 1400 µm were utilized to sieve pellets samples for 20 minutes then, the sample quantity in each sieve was weighed and the yield percentage of sample with size range 710-1120 µm was calculated based on the following equation:

Yield percent = (final pellet weight/initial powder weight) × 100

4.2.4.2. Aspect Ratio

A stereomicroscope (Carl Zeiss, Oberkochen, Germany) and Leica Quantimet 500 C image analysis software (Leica Microsystems, Wetzlar, Germany) were used to measure the aspect ratio based on the following equation:

Aspect ratio = D_{max}/D_{min}

4.2.4.3. Hardness and Deformation Properties

A texture analyzer with probe speed of 20 mm/minutes using measuring force range 0-200 N, with the output of 0–5 V, and sensitivity of ±0.5% ±0.1 digit was utilized to estimate the breaking hardness and deformation behavior of pellets prepared by direct pelletization according to DoE beside layered pellets at different molar ratios (2:1, 1:1 and 1:2) then mean and standard deviation of breaking hardness of all pellets were estimated.

4.2.5. API-Loaded Pellet Test

4.2.5.1. Electrokinetic Potential properties

The surface electrokinetic zeta potential of hydrochlorothiazide and amlodipine besylate pellets beside plain pellets that prepared by direct pelletization and pure drugs was measured by a SurPASS 3 Instrument (Anton Paar, Graz, Austria) in distilled water and PBS buffer. All samples were measured at 20 consecutive points with stabilization of the zeta potential value in a powder cell at an adjustable part in contact with the continuously flowing liquid medium.

4.2.5.2. Dissolution Test

Ereweka DT 700 dissolution apparatus (Erweka DT700 LH, Germany) was used to measure drug release of pellets containing amlodipine besylate and hydrochlorothiazide. Phosphate buffer (pH = 6.8) and HCl (pH 1.2) were applied as dissolution media with a total volume of 900 mL at a temperature of 37 ± 0.5 °C and a rotation speed of 100 rpm. After that, 3 mL were removed at different time intervals (5, 10, 15, 20, 30, 45, 60, 75, 90, and 100 minutes) for hydrochlorothiazide pellets and (5, 10, 15, 20, 30, 45, and 60 minutes) for amlodipine besylate pellets. The UV–VIS spectrophotometer (Unicam Helios α, Spectronic Unicam, Budapest, Hungary) was used to measure absorbance at 272 nm and 238 nm for hydrochlorothiazide and amlodipine besylate pellets, respectively. Then, the drug release percent was measured based on the amlodipine besylate calibration curve equation (Absorbance=0.0426X) with a correlation coefficient R^2 of

0.9991, whereas the hydrochlorothiazide calibration curve equation (Absorbance=0.0139X−0.0066) had a correlation coefficient R^2 of 0.9992.On the other hand, the cumulative release percent of layered pellets that contain different molar ratios of amlodipine besylate and hydrochlorothiazide (2:1, 1:1, 1:2) was estimated using the same dissolution apparatus in 900 mL of HCl (0.1N) media using the same wavelengths and calibration curve equation

4.2.5.3. Fourier Transform-Infrared Spectroscopy (FTIR)

FTIR spectra Avatar330 FTIR (Thermo Fisher Scientific Inc., Waltham, MA, USA) was applied to analyse different samples involving microcrystalline cellulose, mannitol, polyvinyl pyrrolidone, pure drugs, amlodipine besylate and hydrochlorothiazide pellets that prepared by direct pelletiztion beside layered pellets that contain drugs combination at different molar ratios (2:1, 1:1 and 1:2). The measurement was performed after forming a compact disk of sample with potassium bromide (0.2 g) at wavelength range 400–4000 cm^{-1}, spectral resolution of 4 cm^{-1}, 128 scan, CO_2 and H_2O corrections.

4.3. Layering pelletization

4.3.1. Preparation of co-amorphous preparation

Different molar ratios of pure amlodipine besylate and hydrochlorothiazide were blended by a mortar and pestle, then it was mixed with 54 g of cellets containing MCC and 0.6 g of talc in the ProCepT 4M 8 granulator (ProCepT NV, ZelZate, Belgium) with a special temperature jacket (60 °C) at high impeller speed (1500 rpm) for 180 minutes. The resulting pellets were sieved through a 50-mesh screen (Retsch GmbH, Haan, Germany) and then they were stored in a freezer (−20 °C) for further investigation.

4.3.2. Characterization of layered pellets

4.3.2.1. Differential scanning calorimetry (DSC)

10 mg of all prepared samples were put in sealed aluminium pans with 2 holes on top (100 μL) and inserting them into the calorimeter whereas the empty pan was used as the reference at a temperature between 25-300 °C and a heating rate of 10 °C/min with anhydrous nitrogen at flow rate 50 mL/min then all results were normalized and analyzed by STARe SW 16.30 software.

4.3.2.2. X-ray powder diffraction (XRPD)

10 mg of all samples were analysed using a BRUKER D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) with slit-detector Cu Kλ1 radiation (λ = 1.5406 Å) at spanning

an angular range of 3–60° 2θ at step time of 0.1 s, step size of 0.007° and voltage of 40 kV and 40 mA. The results were analysed and their crystallinity index was recorded in DIFFRAC.EVA.V5.2 software.

4.3.2.3. Micro-computed tomography (micro-CT) measurements

The layered pellets morphological features were determined by scanning of samples using high-resolution computed tomography (TESCAN UniTOM XL Spectral, TESCAN, Czech Republic) with an open-type pumped X-ray source, 15 W tube power, 3 µm pixel resolution, an exposure time of 255 ms. H, 70 kV tube, 360° sample rotation, 0.125° rotation step and scan time of 39 minutes. After reconstruction of the images with Panthera 1.6 (TESCAN, Czech Republic) software, the volume-rendered 3D CT images were recorded using the same software, then "Paint and Segment" method in VGSTUDIO MAX 2023.4 (Volume Graphics) was used to obtain AI segmented images of the layered pellets.

4.3.2.4. Stability study

All samples were placed in a desiccator filled with freshly activated silica at a temperature of 25 °C under dry conditions. Then, XRPD was used to estimate the crystallinity index after 1 month.

5. RESULTS AND DISCUSSION

5.1. Direct pelletization

5.1.1. Risk Assessment

Risk assessment is considered a good additional option that provides an in-depth understanding of a huge number of variables that affect different pelletization process steps beside contributing to the appropriate selection of QTPPs, CQAs, CPPs and CMAs by applying different methods such as Ishikawa diagram, Pareto chart and risk estimation matrix to obtain the required pellet quality.

5.1.1.1. Ishikawa Diagram

The fishbone diagram (*Figure 1*) divided all process variables that could affect the manufacturing of pellets with desired quality into four categories, involving process parameters, product characteristics, material attributes and the therapeutic goal.

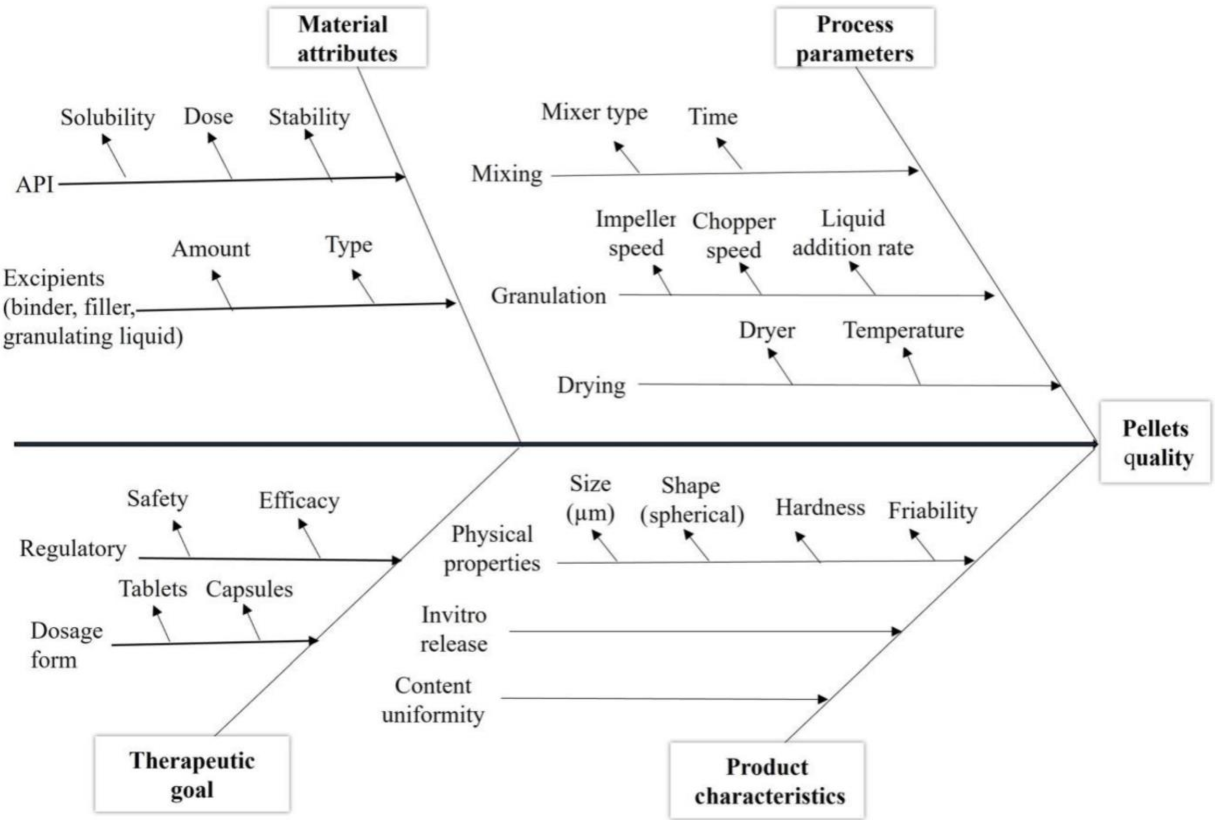

Figure 1. *Ishikawa diagram for determining critical factors for direct pelletization by using Pro-CepT granulator*

5.1.1.2. Risk Estimation Matrix and Pareto Chart

Based on the summary of all process variables, CQAs and QTPPs were identified (*Table 5* and *Table 6*), then risk assessment estimation matrix provided a qualitative evaluation of the relationship between QTPPs and CQAs in addition to the relationship between (CPPs)/(CMAs) and (CQAs) in three grades: high (H), medium (M) and low (L) (*Figure 2*) according to preformulation studies, literature review and group experiments.

Table 5. *Quality target product profile (QTPP) of direct pelletization with ProCepT granulator*

QTPP	Goal	Justification
Morphological feature	Spherical particles with a narrow size distribution	To enhance flow properties during the manufacturing process, solubility, and release kinetics of the drug [52,55].
Mechanical properties	High tensile strength Low friability	Pellets must be able to withstand mechanical forces during various technological processes, such as filling and coating [37].
Efficacy	Optimum content uniformity	Multiparticulate particles are freely distributed in the gastrointestinal tract, which leads to enhanced absorption, in addition to the control of drug release according to the required purpose [52].
Safety	Low toxicity	Small particle size reduces dose dumping and, consequently, gastrointestinal tract irritation and drug toxicity [55,57].
Dosage form	Capsule and tablet	Most applicable dosage forms due to accuracy, stability, and patient compliance [58]

Table 6. *Critical quality attributes (CQAs) of pellets of direct pelletization*

CQA	Goal	Justification
Size	500–1500 μm	To minimize segregation hazard and better coating [52,53].
Aspect ratio	<1.2	Spherical particles have good flow properties, which is a critical factor in the preparation of solid dosage forms [38].
Hardness	Within a good range	To ensure good tableting compression, capsule-filling, and coating [38,54].
Friability		
Angle of repose	25–40	Good rheological properties can be used as an indicator of the degree of sphericity. On the other hand, flow properties with compressibility behavior are very important for the direct compression of tablets and filling of capsules [52,55].
Hausner factor	1–1.34	
Drug release percent	>80%	To ensure high drug absorption and bioavailability [56]
Content uniformity	Within the required range	To obtain an optimum therapeutic effect with the lowest toxicity and side effects [3].

a.

CQAs	QTPPs				
	Safety	Efficacy	Physical feature	Mechanical properties	Dosage form
Aspect ratio	H	H	H	L	H
Yield percent	H	H	H	L	H
Hardness	L	M	M	H	H
Drug release	H	H	L	L	H
Content uniformity	H	H	L	L	H
Friability	L	M	M	H	M

b.

CQAs	CPPs/CMAs										
	Granulation							Drying		Mixing	
	Binder type	Binder amount	Liquid volume	Filler type	Impeller speed	Chopper speed	Addition rate	Dryer type	Drying temperature	Mixing time	Mixer type
Aspect ratio	M	M	H	M	H	H	H	M	M	L	L
Yield percent	M	H	H	M	H	H	H	L	L	M	M
Hardness	H	H	H	L	M	L	L	M	M	L	L
Drug release	M	M	M	H	H	H	M	M	M	L	L
Content uniformity	L	L	M	L	M	M	L	H	H	H	H
Friability	H	H	H	L	M	L	L	M	M	L	L

Figure 2. *Risk estimation matrix* ***A****. Effect of quality target profile (QTTP) with critical quality attributes (CQAs),* ***B****. Critical quality attributes (CQAs) with critical material attributes (CMAs)/critical process parameters (CPPs) using three grades: red color—high (H), yellow color—medium (M), and green color—low (L) for direct pelletization technique.*

Based on the risk estimation matrix and the Pareto chart (*Figures 2 and 3*), five factors were estimated as independent variables in DOE, including chopper speed, liquid addition rate, impeller speed, binder concentration and granulating liquid amount. Also, yield percent, hardness and aspect ratio were estimated as dependent variables in DOE as they had the highest severity score.

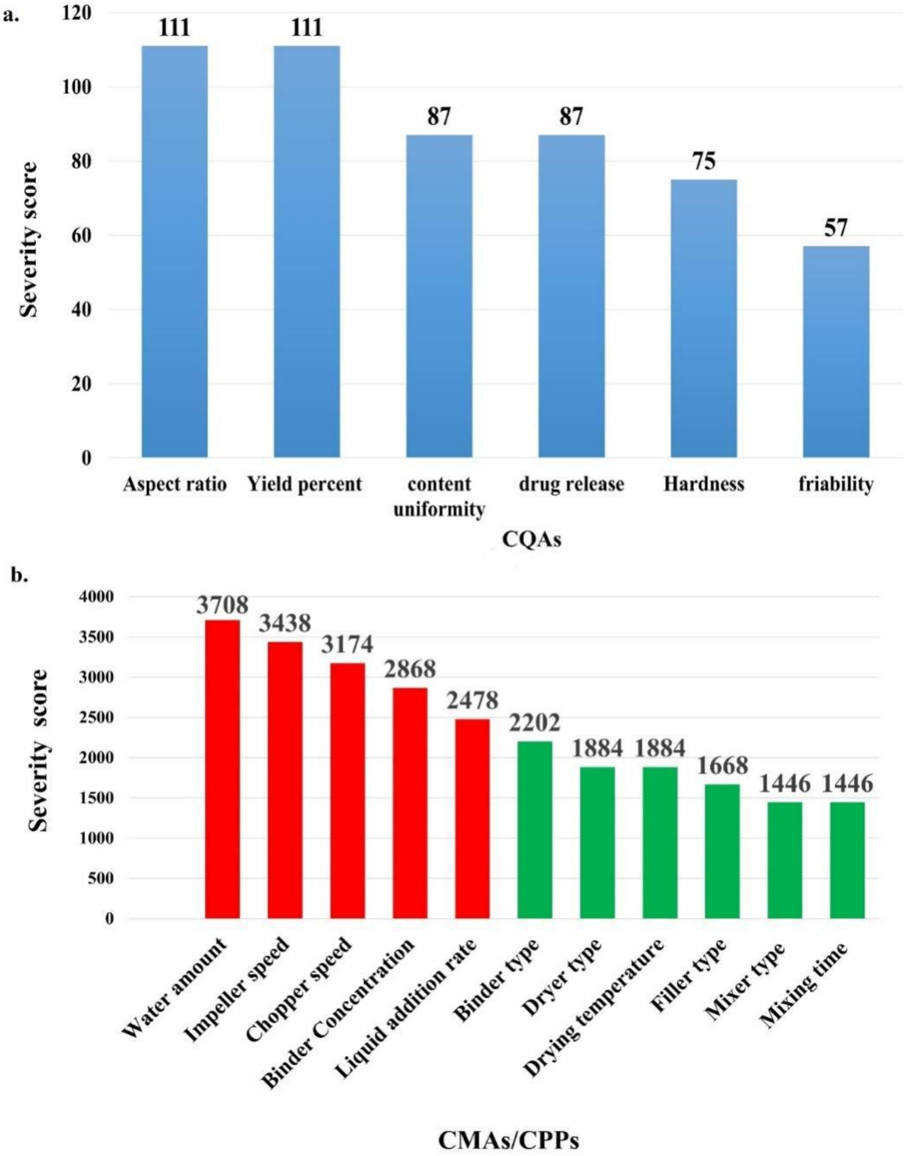

***Figure 3**. Pareto chart of **(a)**. Quality target profile (QTTP) with critical quality attributes (CQAs), **(b)**. Critical quality attributes (CQAs) with critical material attributes (CMAs)/critical process parameters (CPPs) using two grades: red color—high (H), and green color—low (L) for direct pelletization techniques*

5.1.2. Preparation of Pellets

The selection of excipients plays an essential role in the pelletization technique due to the effect of their physicochemical properties on the quality of the produced pellets. Based on previous studies, utilization of fillers like MCC that have an advantageous effect on the pelletization process as its had a plastic texture, cohesive behavior, disintegration ability in small particles, excellent properties of high water uptake and high ability of gel formation during pellets formation. This effect leads to the production of pellets with a smoother surface and a more spherical feature than other fillers [59].

However, applying MCC leads to extending the dissolution time of hydrochlorothiazide (poorly soluble drug) that require the addition of PVP, which has a high disintegration effect leading to enhance dissolution rate, in addition to its binding effect that reduces the amount of utilized water during pellets preparation. Moreover, binders contribute to obtaining particle consolidation and uniform drug distribution, while using a high water-soluble secondary filler like mannitol contributes to reducing bulk density, modifying flow properties and improving drug dissolution [1,60].

5.1.3. Design of the Experiment

ANOVA was applied to estimate the significance of responses in the quadratic model (linear, interactive and polynomial). The *p-values* and the impact of the model variables (R^2, adjusted R^2 and MS residual) on each process variable were estimated at the 95% confidence level. Positive signs indicate an increase in response value, whereas negative signs indicate a reduction in response value.

5.1.3.1. Screening Design

The main advantageous effect of screening design is excluding of independent process variables that had a non-significant effect on dependent variables in addition to providing more information about the effect of variables on process reponses involving aspect ratio (Y_1), yield percent (Y_2) and hardness (Y_3), that are described in *Equations 1-3* (p < 0.05).

$$Y_1=\mathbf{1.626}+\mathbf{0.03025X_1}+\mathbf{0.06325X_2}+\mathbf{0.21375X_3}+\mathbf{0.0465X_4}-0.0025X_5-\mathbf{0.0765X_2X_3} \quad (1)$$

R^2=0.99999, Adjusted R^2=0.99993, MS Residual=0.0000045

$$Y_2=39.25-3.25X_2-22.25X_3+4.75X_4+2.25X_2X_3-1.25X_2X_4 \quad (2)$$

R_2= 0.9979, Adjusted R^2= 0.99265, MS Residual = 4.5

$$Y_3=\mathbf{35.76963}-\mathbf{0.57513X_1}-\mathbf{0.88987X_2}-0.43938X_3+\mathbf{3.01137X_4}-0.18512X_5-\mathbf{1.29713X_2X_4} \quad (3)$$

R^2= 0.99989, Adjusted R^2=0.99922, MS Residual=0.0107311

According to the screening design results (*Table 7*), binder amount and chopper speed were the most significant effects on all dependent variables due to their effect on clumps breaking, size reduction below the required size and enhancement of binder distribution, which subsequently led to the enhancement of hardness, particle growth mechanism and flow properties [2]. Moreover, the volume of the granulating liquid and impeller speed had a significant effect on some variables wheras binder amount had positively affected hardness and yield percent. The performed experiments at the low level of granulating liquid volume (55 mL) exhibited a higher yield percent and lower aspect ratio; however, it did not significantly affect pellets hardness.

On the other hand, it was noticed that the effect of chopper speed corresponds to granulating liquid volume and it found that chopper speed with low granulating liquid volume (55 mL) had negative effect on aspect ratio and hardness but it had positive effect on yield percent due to reducing particle size. In contrast it had an opposite effect at higher granulating liquid volume due to particles aggregation, increasing pellet size more than desired range and loss of spherical shape.

Although addition rate of the granulating liquid had negative effect of the on the hardness and aspect ratio (increase in pellets size and porosity), it did not significantly affect aspect ratio, yield percent and hardness and this investigation is agreed with other studies [61]. Therefore, it was excluded and fixed at a low level (5 mL/minute) while granulating liquid volume, impeller speed, binder amount and chopper speed were selected for more experimental design.

Table 7. *Screening design results*

Experiment No.	Aspect ratio	Yield percent (%)	Hardness (N)
1	1.287	73	41.834
2	1.258	61	32.437
3	1.57	58	37.757
4	1.534	54	32.808
5	1.773	12	32.265
6	1.933	24	40.102
7	1.753	11	33.523
8	1.9	21	35.431

5.1.3.2. Two-Level Full Factorial Design

Applying a two-level full factorial design was essential to accurately estimate the effect of independent variables and their interaction with each other on the dependent variables. Moreover, adding an extra center point experiment to identify the curvature relationship[33]. The relationship between factors and responses (*Table 8*) was analysed and the best-fit *Equations 4–6* for aspect ratio (Y_1), yield percent (Y_2) and hardness (Y_3), respectively ($p < 0.05$), were estimated according to the highest value of R^2 and adjusted R^2 with the lowest MS residual.

$$Y_1=\mathbf{1.629625-0.409625C^*+0.116750X_1+0.020250X_2+0.217125X_4+0.046125X_1X_2+0.0055X_1X_3+0.086X_1X_4+0.04X_2X_3-0.0865X_2X_4-0.13125X_3X_4-0.049125X_1X_2X_3+0.042875X_1X_2X_4+0.029X_1X_3X_4-0.016X_2X_3X_4} \quad (4)$$

R^2=0.99998, Adjusted R^2=0.9998, MS Residual=0.0000223

$$Y_2=\mathbf{38.125+25.875C-4.625X_1+2.5X_2-15.375X_3-11.375X_4-2.125X_1X_3}+0.375X_1X_4+\mathbf{1.25X_2X_3}+2.25X_2X_4+\mathbf{2.625X_3X_4}+0.5X_1X_2X_3+\mathbf{2.25X_1X_2X_4+3.125X_1X_3X_4+1X_2X_3X_4} \quad (5)$$

R^2=0.99987, Adjusted R^2=0.99893, MS Residual=0.5

$$Y_3=\mathbf{34.11465}-2.38456C+\mathbf{0.84919X_1}+\mathbf{1.22056X_2}+\mathbf{1.95581X_3}+\mathbf{1.56156X_4}\mathbf{-0.86731X_1X_2}+\mathbf{1.13919X_1X_4}-0.43431X_2X_3+\mathbf{1.11906X_2X_4}+0.44831X_3X_4+\mathbf{0.99881X_1X_2X_3}+0.36319X_1X_2X_4+0.66369X_1X_3X_4-0.30919X_2X_3X_4 \tag{6}$$

R^2=0.99541, Adjusted R^2=0.96328, MS Residual= 0.5226571

C* the deviation of the response surface from a linear relationship

Table 8. *Two-level full factorial design results*

Experiment No.	Aspect ratio	Yield percent (%)	Hardness (N)
1	1.204	69	30.938
2	1.258	61	32.437
3	1.229	61	30.593
4	1.436	71	30.214
5	1.524	67	32.084
6	1.237	78	34.347
7	1.57	58	37.757
8	1.842	35	32.054
9	1.845	11	30.559
10	2.292	13	31.556
11	1.753	11	33.523
12	2.023	27	37.45
13	1.582	7	31.129
14	1.933	24	40.102
15	1.396	54	39.54
16	1.95	20	41.55
17 (c)	1.22	65	31.73

According to design results, it was found that liquid volume had a significant positive effect on pellet hardness due to increasing cohesive force and consequently particle aggregation, while it was significantly affected by high impeller speed which improves binder distribution of liquid volume during pelletization [9]. Additionally, chopper speed has a positive effect on hardness, which is not in agreement with Kristó et al. and may be due to decreasing the pellet size.

Also, aspect ratio is positively affected by chopper speed, granulating liquid volume, impeller speed and some two-way and three-way interactions. It was found that reducing the granulating liquid lead to a decrease in particles aggregation and particle size distribution, which has an advantageous effect on increasing sphericity and decreasing the aspect ratio < 1.2. In contrast, very high values of impeller speed contribute to improving particle growth, porosity and produce particles with a high aspect ratio (lower sphericity) while high chopper speeds lead to reduce sphericity due to the breakdown of granules [2,59].

Moreover, the yield percent had negatively affected by liquid volume on yield percent, particularly in experiments 13, 11, 10 and 9 that were performed at a high level of liquid volume (60 mL) and low binder amount (1 g). All those experiments produced lower yield percent that were less than 20% due to particles aggregation and over-wetting, subsequently, the enlargement of particles was more than the desired size range. Furthermore, applying a lower binder amount leads to a decrease in yield percent and that may be related to failure in the bridges formation between particles that are important to obtain pellets with desired size. The central point experiment revealed the presence of a significant non-linear relationship (curvature) between the independent variables and two dependent variables (aspect ratio and yield percent).

5.1.3.3. Central Composite Design

The central composite design with minimal extra experiments revealed an additional estimation of the non-linear relationship between independent variables and dependent variables (*Table 9* and 10) [62]. The design results were analysed and the significant coefficients of linear and quadratic models (p < 0.05) were presented in the best-fit second-order *Equations 7–9* of aspect ratio (Y_1), yield percent (Y_2) and hardness (Y_3), respectively.

$$Y_1=1.224271+\mathbf{0.194722X_1}+\mathbf{0.353415X_2^2}+\mathbf{0.107556X_3}+\mathbf{0.049915X_4^2}+\mathbf{0.131125X_1X_2}+\mathbf{0.086X_1X_3}-\mathbf{0.0865X_1X_4}+0.04X_2X_4+0.046125X_3X_4 \quad (7)$$

R^2=0.9141, Adjusted R^2=0.8658, MS Residual=0.0134704

$$Y_2=\mathbf{63.25-14.3333X_1-11.5556X_2-24.8056X_2^2}-\mathbf{5.3333X_3-2.2778X_4}+2.625X_1X_2-2.25X_1X \quad (8)$$

R^2=0.93101, Adjusted R^2=0. 90418, MS Residual=42.43056

$$Y_3=32.7443+\mathbf{1.4023X_1}+0.9498X_1^2+\mathbf{1.9642X_2}-0.9602X_2^2+\mathbf{0.9361X_3+1.34511X_4}+1.4298X_3^2+\mathbf{1.1137X_1X_3+1.1445X_1X_4}-0.8927X_3X_4 \quad (9)$$

R^2=0.83498, Adjusted R^2=0.72496, MS Residual=2.919908

Table 9. *Central composite results*

Experiment No.	Aspect ratio	Yield percent (%)	Hardness (N)
17	1.184	73	33.96
18	1.215	62	33.81
19	1.456	14	29.74
20	1.667	35	34.21
21	1.333	61	30.914
22	1.183	65	35.19
23	1.220	69	32.53
24	1.288	94	36.2
25	1.252	64	32.85
26	1.219	67	31.26

Despite a reduction in coefficient values of the central composite design, it showed a more obvious explanation about the quadratic and linear interaction between independent variables and dependent variables [33,34]. Based on the central composite design, impeller speed (X_1) and the volume of the granulating liquid (X_3) have a significant effect on all independent variables (*Table 10*). Moreover, the linear chopper speed (X_2) does not affect the aspect ratio, whereas the non-linear or quadratic chopper speed (X_2^2) had a positive effect on the aspect ratio in addition to the negative effect of linear and quadratic chopper speed on yield percent.

On the other hand, linear binder concentration (X_4) had a negative effect on the yield percent, while quadratic binder concentration (X_4^2) had a positive effect on the aspect ratio. These results confirmed the presence of significant curvature in the results of the two-level design. Furthermore, the interaction between impeller and chopper speed (X_1X_2) has a significant positive effect on aspect ratio, similar to the individual effects of both variables. Therefore, simultaneous use of high levels of impeller and chopper speeds leads to an increased aspect ratio and reduced sphericity (*Figure 4*).

Table 10. *The coefficients for the X values in the central composite design*

Coefficients for X Values	**Y_1**	**Y_2**	**Y_3**
X_1	**0.194722**	**−14.3333**	**1.4023**
X_2	-	**−11.5556**	**1.9642**
X_3	**0.107556**	**−5.3333**	**0.9361**
X_4	-	**−2.2778**	**1.34511**
X_1^2	-	-	0.9498
X_2^2	**0.353415**	**−24.8056**	0.9602
X_3^2	-	-	1.4298
X_4^2	**0.049915**	-	-
X_1X_2	**0.131125**	2.625	-
X_1X_3	**0.086**	-	**1.1137**
X_1X_4	−0.0865	−2.25	1.1445
X_2X_4	0.04	-	-
X_3X_4	0.046125	-	−0.8927

Note: Significant factors are in bold.

Based on central composite results, the aspect ratio was proportionally affected by linear granulating liquid volume, quadratic chopper speed and linear impeller speed. These results agreed with the two-level design results.

In the other country, the yield percent was negatively affected by linear granulating liquid volume, linear impeller speed, linear and quadratic chopper speed, due to low chopper speeds leading to a reduction in particle size with a narrow distribution, whereas applying a very high value of chopper speeds contributes to the breaking down of particles [6,63]. Additionally, hardness was significantly affected by linear impeller speed, linear binder amount, linear liquid volume and linear chopper, which also confirmed the results of the two-level design (non-significant curvature or the absence of a non-linear relationship) for the same reasons as mentioned in the two-level design.

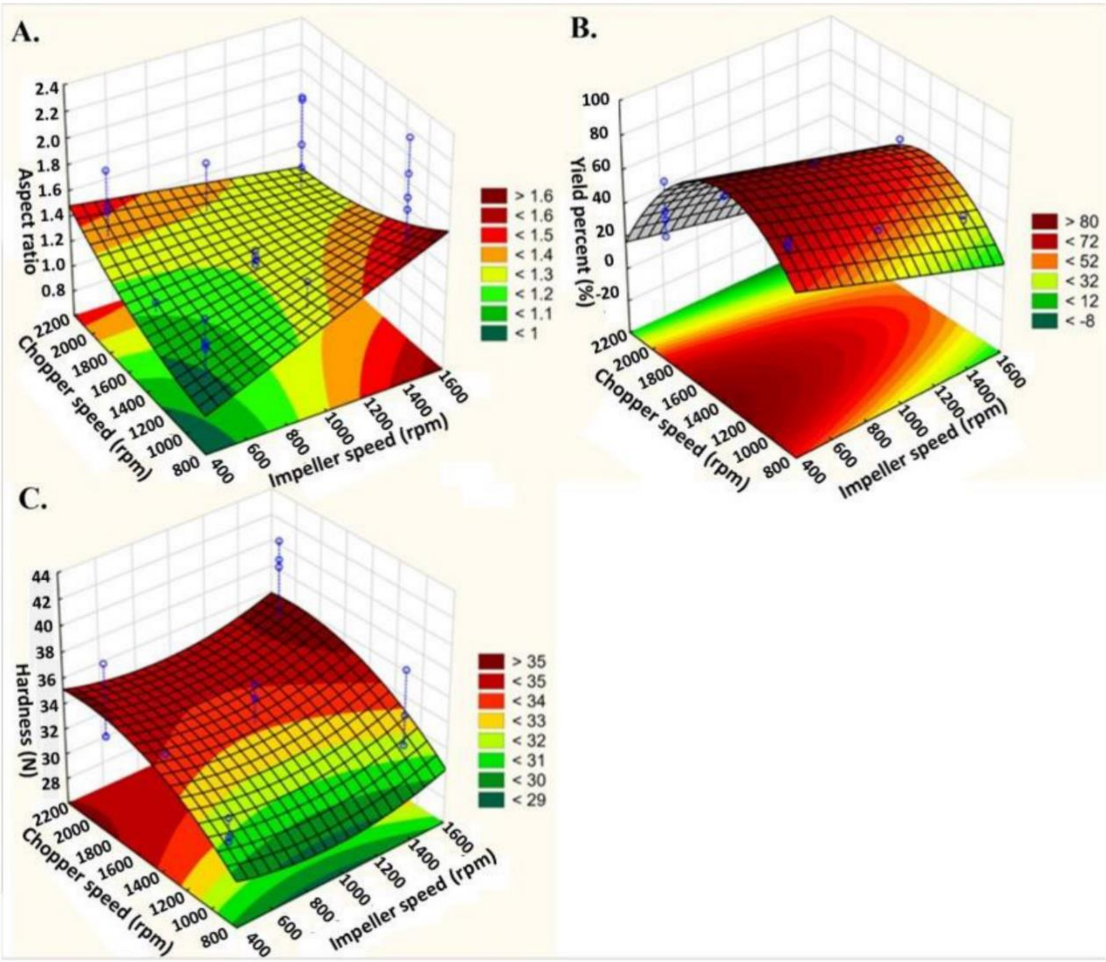

Figure 4. *Three-dimensional response surface plots showing the effects of impeller speed and chopper speed at constant liquid volume (55 mL) and binder amount (2 g) on (A) aspect ratio, (B) yield percent, and (C) hardness*

5.1.3.4. Process Optimization and Validation

A design space was constructed to formulate pellets with desired CQAs, involving yield percent > 80%, hardness < 33 N and aspect ratio < 1.2 (*Figures 4 and 5*). Chopper speed and impeller speed were selected as the main variables (X, Y axis) in the design space due to their higher significant *p-values* and effects on the size of the design space than granulating liquid volume and binder amount.

In spite of a positive effect of granulating liquid volume on hardness, it was fixed at a low level (55 mL) because it had a negative effect on sphericity (aspect ratio > 1.2) and yield percent, whereas binder amount was fixed at a center level (2 g) due to its effect on increasing the size of the design space.

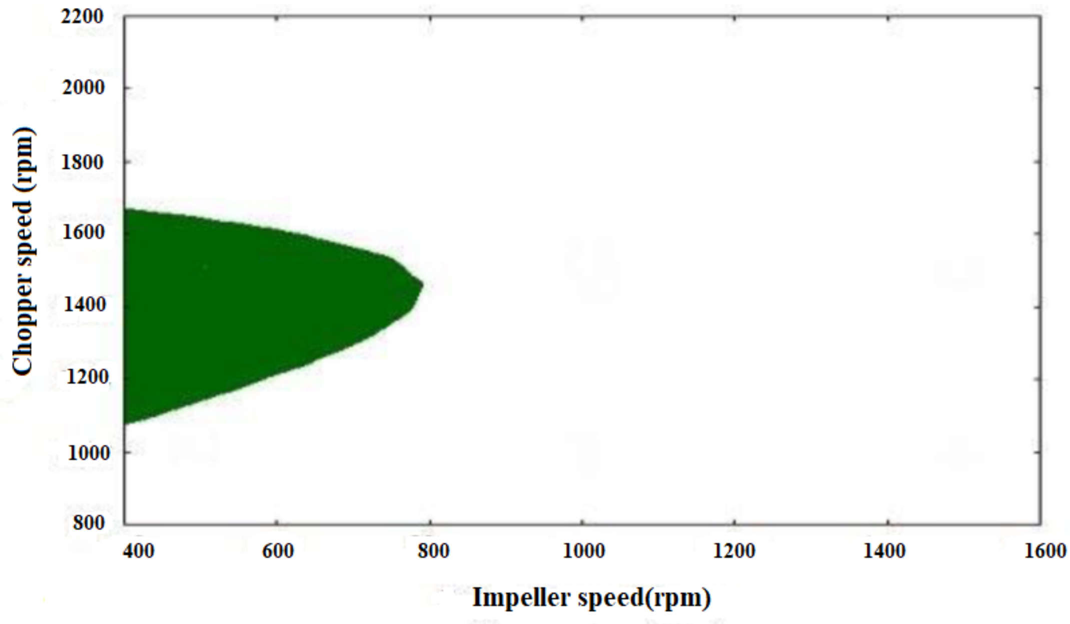

Figure 5. *Design space for direct pelletization with Pro-CepT granulator*

Three different experimental points within the design space were selected to check the validity of the model and it revealed the robustness of the design space as validation results within the desired ranges with a small difference between the practical and predicted results (*Table 11*).

Table 11. *Direct pelletization process validation at fixed binder amount (2 g) and liquid volume (55ml) (EV expected value; PV practical value)*

Exp. No.	Impeller speed (rpm)	Chopper speed (rpm)	Aspect ratio		Yield percent (%)		Hardness (N)	
			EV	PV	EV	PV	EV	PV
1	500	1500	1.204	1.192	87.31	83.36	34.99	34.72
2	600	1300	1.16	1.14	84.73	80.02	34	34.02
3	700	1200	1.204	1.23	80.83	77.93	33.43	33.55

5.1.4. API-Loaded Pellet Test

5.1.4.1. Physical properties

Amlodipine besylate and hydrochlorothiazide containing pellets were prepared using the selected optimized experiment condition, including chopper speed of 1500 rpm, impeller speed of 500 rpm, granulating liquid volume of 55 mL and binder amount 3 g. (*Table 12*).

Table 12. *Physical test results of amlodipine besylate and hydrochlorothiazide*

Test	Amlodipine besylate	Hydrochloro-thiazide	Drug-free pellet
Aspect ratio	1.14	1.19	1.192
Yield percent (%)	82	79	83.36
Hardness (N)	35.89	37.86	34.02

The effects of the addition of amlodipine besylate and hydrochlorothiazide (model drugs) on the CQAs of the produced pellets, specifically aspect ratio, hardness and yield percent was evaluated using a t-test (*Figure 6*). It was showed that the addition of both model drugs had no significant on hardness and aspect ratio (p value < 0.05) of the optimized formula. On the other hand, hydrochlorothiazide had a significant effect on the yield percent due to the higher drug amount of hydrochlorothiazide which displaced mannitol and microcrystalline cellulose more than amlodipine besylate beside to the hydrophobic nature of hydrochlorothiazide that may decrease wettability of the powder mixture by granulating liquid and consequently decrease pellets formation and the yield percent [1].

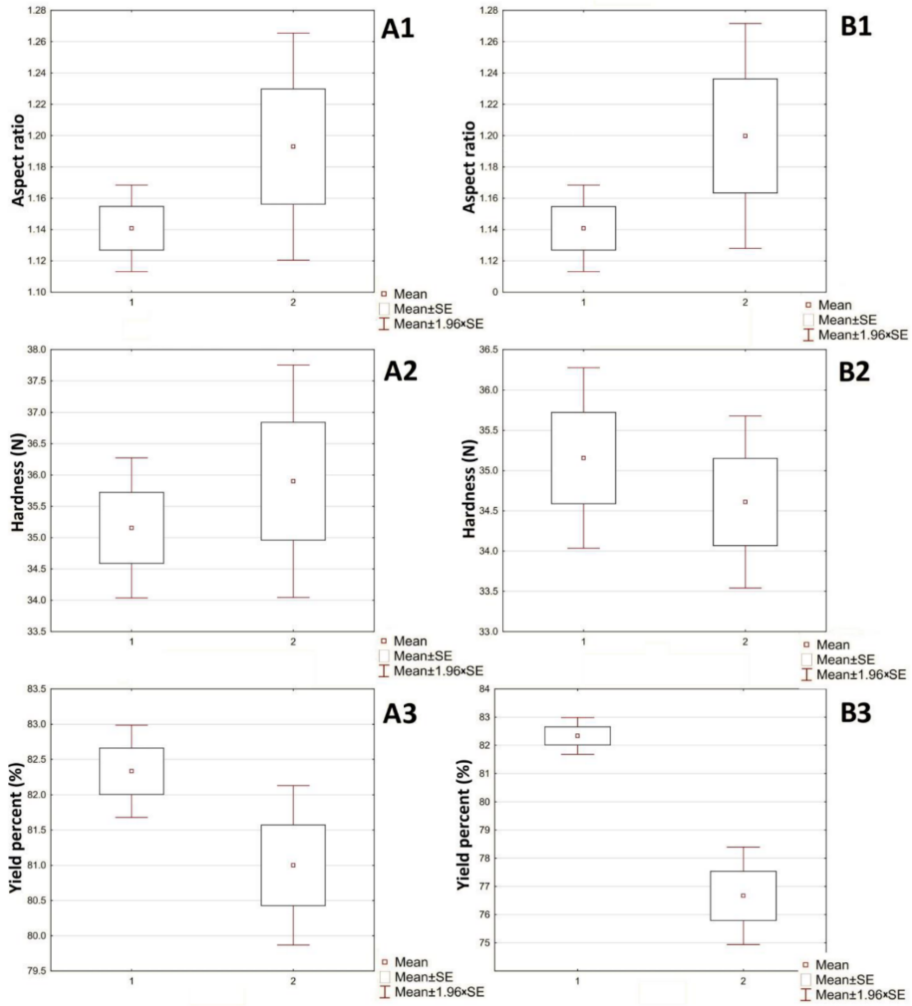

Figure 6. *T-tests of the physical properties of drug-free pellets: (A1–A3) Amlodipine besylate pellets; (B1–B3) Hydrochlorothiazide pellets*

5.1.4.2. Dissolution Test

According to the dissolution profile (*Figure 7*), the cumulative release percent of amlodipine besylate and hydrochlorothiazide is 92.6 ± 1.6% / 45 minutes and 80.3 ± 2.08% / 100 minutes, respectively. It found that a multiparticulate system enhanced drug dissolution due to enhancing the pellets wettability due to the utilization of polyvinylpyrrolidone which has a hydrophilic nature. Also, utilization of trehalose excipient contributes to enhancing the microporosity of the formulated pellets in addition to formation a glass solution with the hydrophobic drug that improved drug dissolution behavior [60].

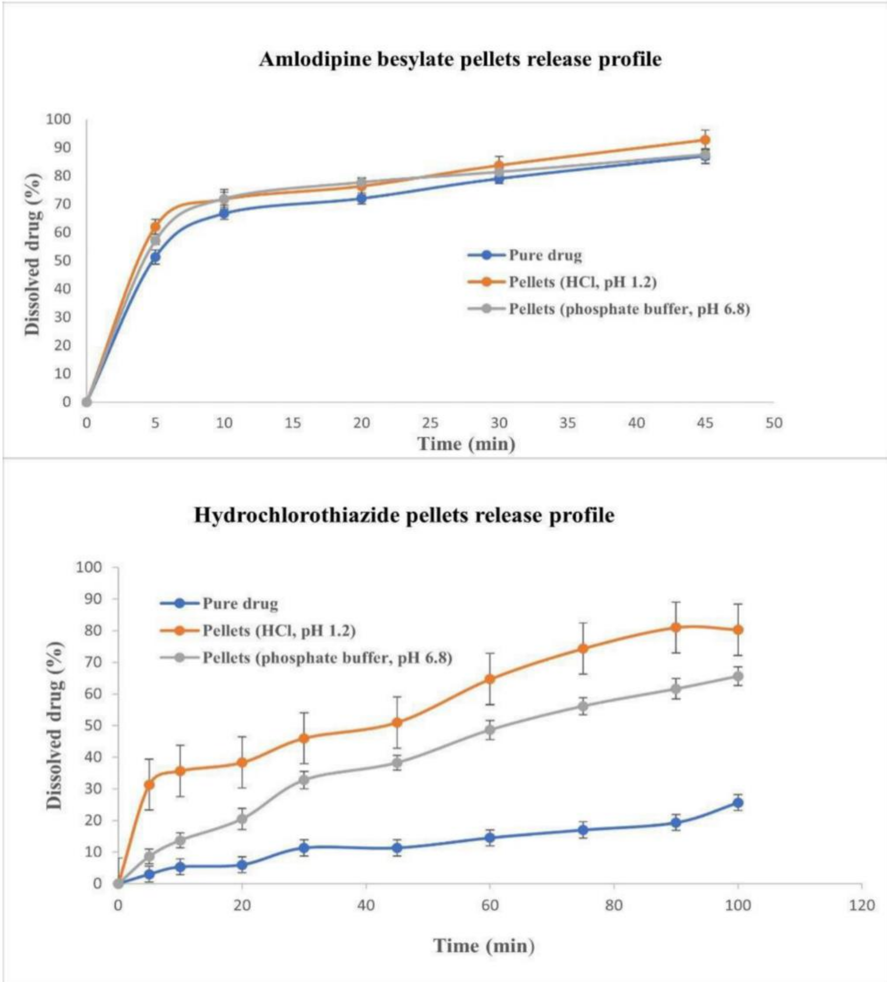

Figure 7. *Dissolution release profile for amlodipine besylate pellets and hydrochlorothiazide pellets*

The zeta potential of pellets at the interface of solids/liquids were measured using flow potential in side SurPass apparatus that accurately estimates lower zeta potential (few millivolts) in addition to surface charge information.

According to the measurement results (*Figure 8*), it was noticed that Vivapur powder had the longest measurement time due to it particle size (60 µm), that forms a compact structure in the apparatus cell and led to retard flow of PBS buffer. In contrast, the flow of PBS buffer through pellets was very easy and fast as it had a large particle size and empty space between the pellet in the cell. After that, the pellets were slowly disintegrated when buffer passed through it however, fluid flow did not slow down as the cell volume did not change.

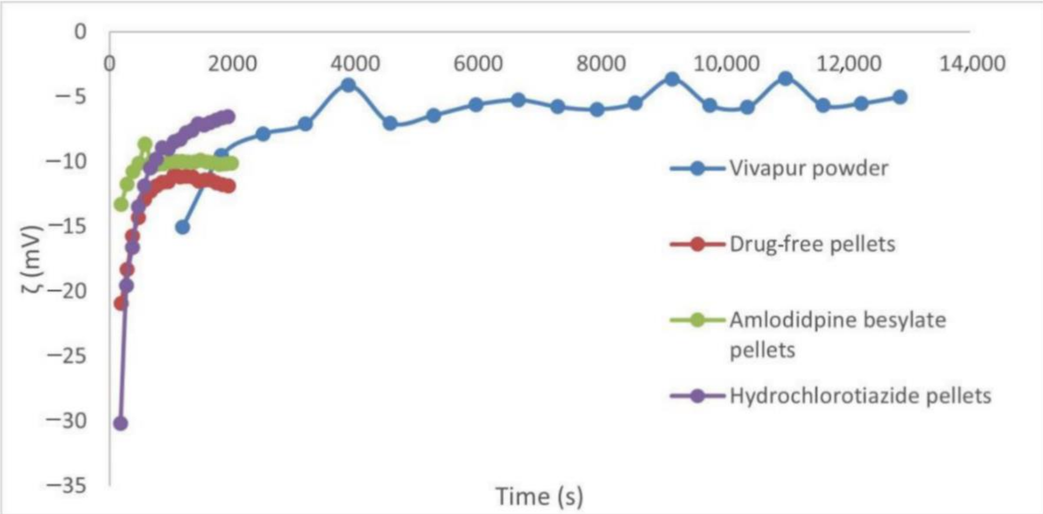

Figure 8. *Results of electrokinetic potential measurements (SurPass measurements)*

Moreover, amlodipine besylate pellets exhibited a small change in the zeta potential value whereas hydrochlorothiazide exhibited a higher value than plain pellets (*Table 13*). These results were consistent with the dissolution results (*Figure 7*), due to the similarity of the dissolution curves for amlodipine besylate. In contrast, the dissolution of hydrochlorothiazide pellets was significantly enhanced.

The hydrophilic and lipophilic characteristics in addition to the electrostatic interactions play a very important role in dissolution behavior due to effect of the drugs adsorption on the MCC surface on dissolution profile, particularly amlodipine besylate had higher affinity to the negatively charged cellulose surface [64] while hydrochlorothiazide has a higher affinity for positively charged micelles [65].

Also, hydrochlorothiazide had almost similar zeta potential value to Vivapur, while, amlodipine besylate pellets had a similar zeta potential value to pellets without drug that had some interactions with the excipients.

Furthermore, amlodipine besylate can also interact electrostatically with MCC and it did not improve the release percentage. Therefore, electrokinetic potential estimation is promising as the dissolution kinetics are affected by the electrostatic and adsorption properties of solid surfaces**.**

__Table 13.__ Results of SurPass measurements in PBS buffer

Sample	Zeta potential (mV)
Vivapur powder	−5.01
Drug-free pellets	−11.87
Amlodipine besylate pellets	−10.13
Hydrochlorothiazide pellets	−6.55

5.1.4.3. FTIR spectroscopy

FTIR spectroscopy was performed to investigate the chemical interactions of drugs with other excipients (*Figure 9*). For the amlodipine besylate pellet sample, the two peaks displayed medium intensities between 3300 and 3400 cm^{-1} and the single peak between 3200 and 3300 cm^{-1} indicates the presence of the stretching of primary and secondary amines, while the peak appearing at 3400 cm^{-1} is related to the OH groups of mannitol and microcrystalline cellulose. The aliphatic CH_3 stretching was detected between 2800 and 3000 cm^{-1}. The overlapping peak between 1600 and 1800 cm^{-1} resulted from aromatic skeletal stretch, the ester carbonyl group of the drug in addition to the aromatic ketone of the polyvinyl pyrrolidone polymer. On the other hand, the peak above 1400 cm^{-1} can be attributed to CH_3-CH_2 bending. The absorption peaks of aromatic C-Cl and asymmetric sulfonate (S=O) stretching appeared at around 1000 and 1207 cm^{-1}, respectively [66].

For the hydrochlorothiazide pellet, the same peaks of the hydroxyl group of mannitol and microcrystalline cellulose appeared at 3400 cm^{-1}. Also, symmetric aliphatic CH_2, a doublet primary amine and a single peak of secondary amines were found between 2900 and 3000 cm^{-1} as well as 3300 and 3200 cm^{-1}. Furthermore, the stretching of heterocyclic sulphonate and asymmetric sulphonate appeared between 1000 and 1200 cm^{-1}, while CH_3-CH_2 bending and a C-Cl peak were located at 1400 cm^{-1} and 850 cm^{-1} [67]. All these results suggest that no interaction can be found between the different active ingredients and the excipients. Basically, the characteristic peaks of the excipients are visible, while the peaks of the active ingredient are less visible in the pellets due to the low concentration of the active ingredient.

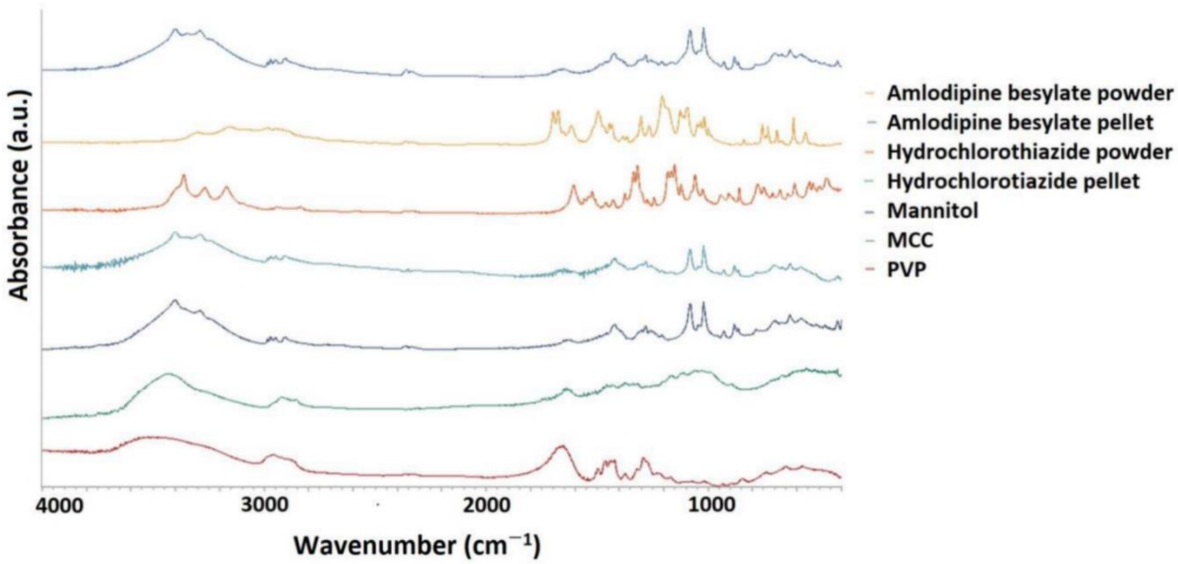

Figure 9. *FTIR spectroscopy of amlodipine besylate pellets, hydrochlorothiazide hydrochloride pellets, and excipients (MCC, PVP and mannitol)*

5.2. Layering pelletization

5.2.1. Differential scanning calorimetry (DSC)

The DSC was performed to estimate the thermal behaviour of producing pellets (*Figure 10*). The crystalline nature of pure amlodipine and hydrochlorothiazide was confirmed based on the presence of a sharp endothermic peak at 199 °C and 274 °C, respectively.

Also, all pellets containing different molar ratios (1:1, 1:2 and 2:1) showed the conversion of crystalline amlodipine besylate to amorphous form due to the absence of its endothermic melting peak. However, hydrochlorothiazide was partially amorphized (physical form change) due to reducing and broadening of its endothermic melting peak [68,69].

Additionally, the melting peak intensities of hydrochlorothiazide in all pellets were estimated [70]. The pellets containing the highest amount of amlodipine besylate (2:1) exhibited the lowest integral of the melting peak (0.88) compared to other pellets (1:1 and 1:2) that exhibited 1.13 and 1.64, respectively. This indicates that increasing amlodipine besylate, that a flexible and large structure, may lead to reduced packing and destroy crystalline form and enhance amorphization of hydrochlorothiazide. On the other hand, it contributes to enhancing the formation of hydrogen bonds that correlate to the increase of amino and ester groups (donor and acceptor groups).

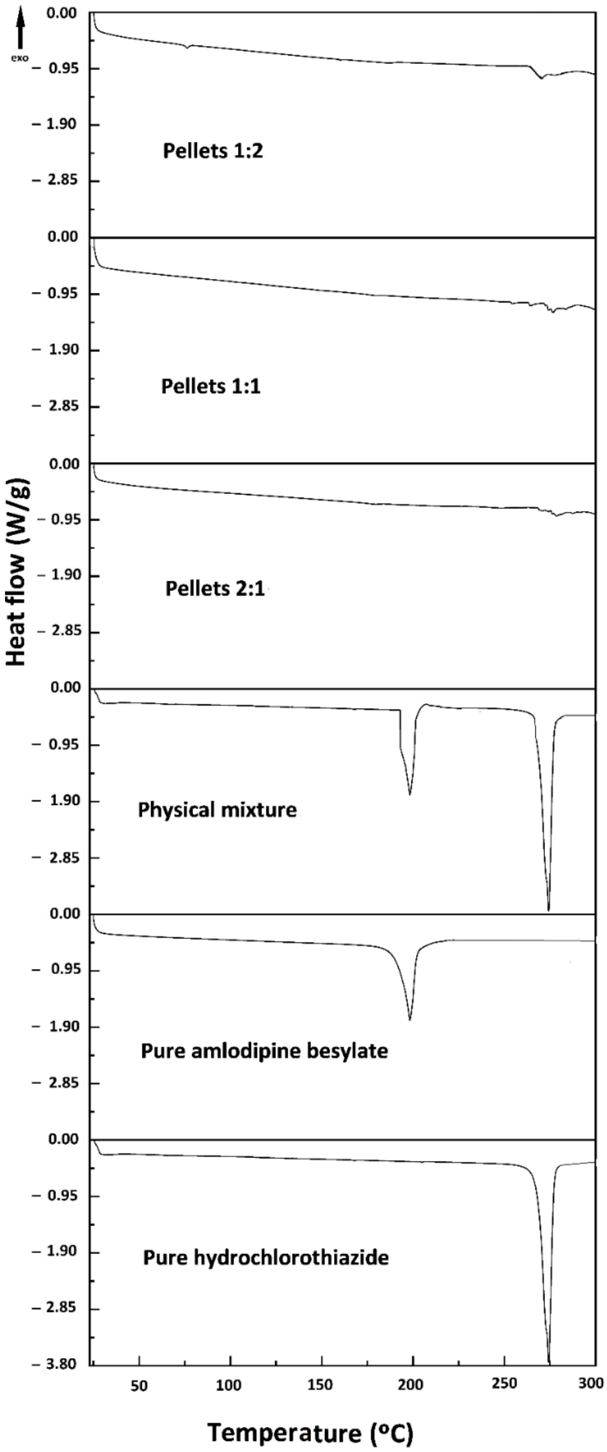

Figure 10. *DSC thermograms of pure drugs and layered pellets containing different molar ratios*

5.2.2. X-ray powder diffraction (XRPD)

According to the diffractogram results (*Figure 11*), it was found that pure amlodipine besylate, hydrochlorothiazide and physical mixture had a crystallinity index 88%, 92.5% and 90.2%, respectively (*Table 14*) with many diffractions that indicate both drugs have a crystalline structure. On the other hand, all producing layered pellets showed lower total net area and diffraction peak intensities [68,69] than pure drugs and physical mixture especially pellets with a molar ratio of 2:1 that had minimum crystallinity index (26.8 %) whereas, the layered pellets with molar ratio a 1:2 and 1:1 had a crystallinity index of 53.6 % and 33%, respectively (*Table 14*).

Increasing the amount of amlodipine besylate in formulated pellets contributes to decrease the crystallinity index and improving amorphization (XRPD results) that agrees with decreasing the integral of the melting endothermic peak and partial amorphization of hydrochlorothiazide (DSC results).

Table 14. *Crystallinity index of the samples*

Sample	Crystallinity index (%)
pure amlodipine besylate	88.0
pure hydrochlorothiazide	92.5
physical mixture	90.2
1:1 pellet	33.0
2:1 pellet	26.8
1:2 pellet	53.6

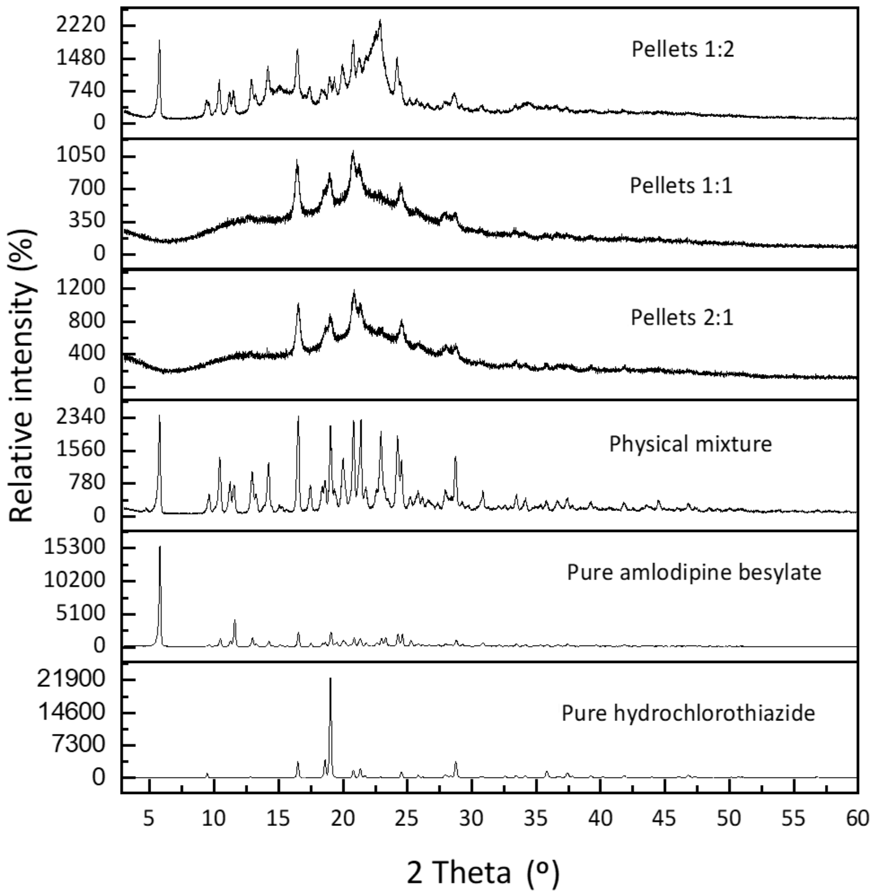

Figure 11. *XRPD diffractogram of layered pellets*

5.2.3. Micro-CT measurements

The internal structure and morphological features of layered pellets were checked by micro-CT (*Figure 12*), including the diameter of the entire pellets and pellet cores [37,71], in addition to structural integrity and layer uniformity through the resulting segmented images[72].

The diameter of formulated layered pellets had higher diameters (1.335, 1.282 and 1.314 mm for pellets with molar concentration 1:2, 1:1 and 2:1) than cellets containing MCC (1.159 mm) and micro-CT images of all formulated pellets confirmed this result due to the complete covering of MCC cellets by a distinguishable thin layer of drug mixture powder.

In other countries, the presence of some voids and cracks that appeared in the core of layered pellets was irrelevant to the applied physical force of the impeller during the pelletization process as it appeared in cellets containing MCC.

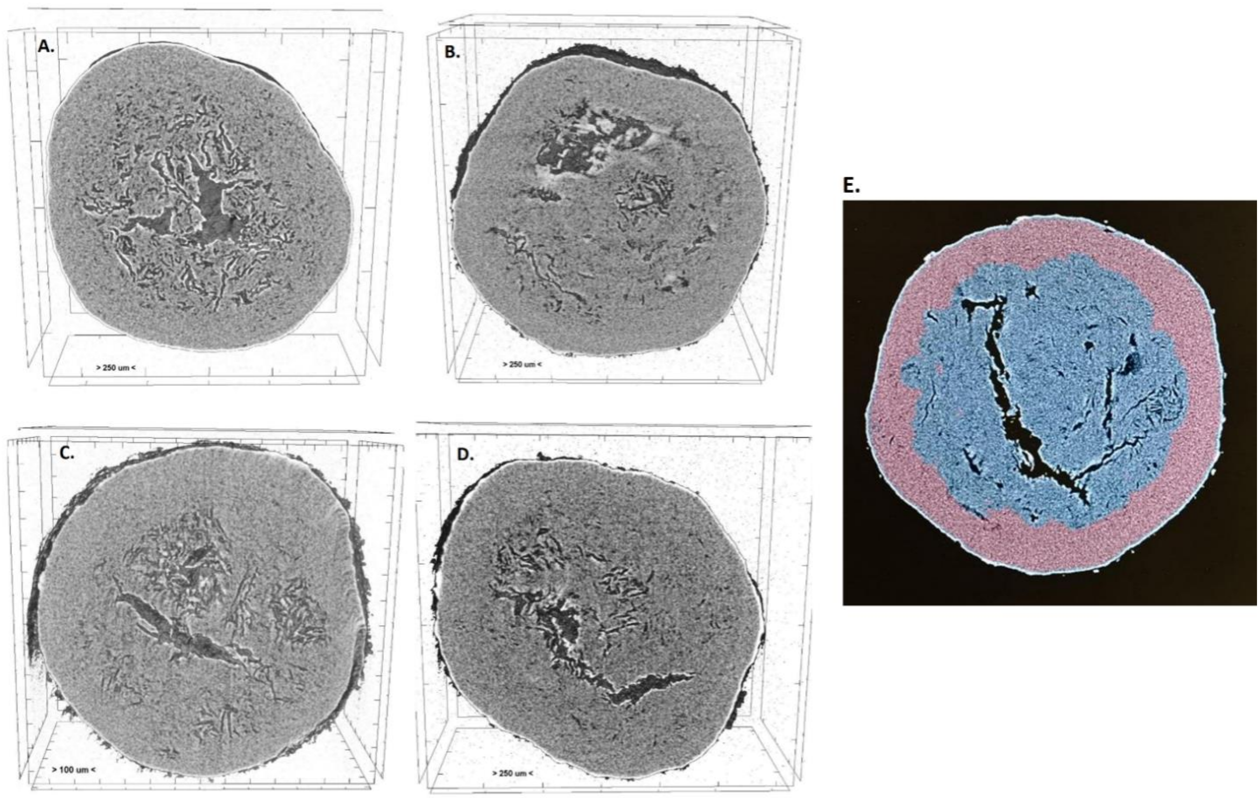

Figure 12. *Micro-CT images of A. pure cellet, B. pellet molar ratio 2:1, C. 1:1, D. 1:2 and E. segmented image of pellet molar ratio 1:2.*

5.2.4. Hardness and mechanical properties

Despite the variation of breaking hardness of layered pellets with cellets (*Table 15*), there was no significant difference between them ($p > 0.05$) and this result agreed with the micro-CT results that mentioned the thickness values of all formulated layered pellets were closer to each other.

Table 15. *Breaking hardness of cellets and different molar ratio API layered pellets*

Experiment No.	Pure cellets	Pellets 2:1	Pellets 1:1	Pellets 1:2
1	20.23	21.3	21.64	25.47
2	17.1	24.7	27.67	21.4
3	17.04	21.51	17.55	25.03
4	17.82	22.75	17.07	16.97
5	17.05	24.6	21.99	18.1
6	16.1	23.53	25.39	23.13
7	18.08	23.86	18.96	24.42
8	19.18	25.9	24.86	24.21
9	24.46	17.93	27.46	17.08
10	22.87	26.39	21.76	22.8
11	24.2	21.99	19.98	22.74
12	21.34	23.6	18.24	17.16
13	23.04	21.94	20.08	16.28
14	18.62	16.4	23.79	22.4
15	19.27	21.16	21.59	22.19
16	20.09	22.95	16.32	24.2
17	17.67	16.9	22.94	16.09
18	22.9	20.24	22.52	24.9
19	23.8	17.19	20.01	26.1
20	16.56	17.3	19.58	25.73
Mean	**19.871**	**21.607**	**21.47**	**21.82**
SD	**2.8**	**3.07**	**3.22**	**3.51**

The addition of a drug mixture layer on the surface of the cellets contributes to increasing breaking hardness due to the high mixing effect of impeller during layering pelletization. This effect led to an increase in the adhesion force between drugs mixture powder and cellets, which consequently enhanced the incidence of surface irregularities and pores filling by drug mixture powder beside slight improving the density and breaking hardness of the formulated pellets [73].

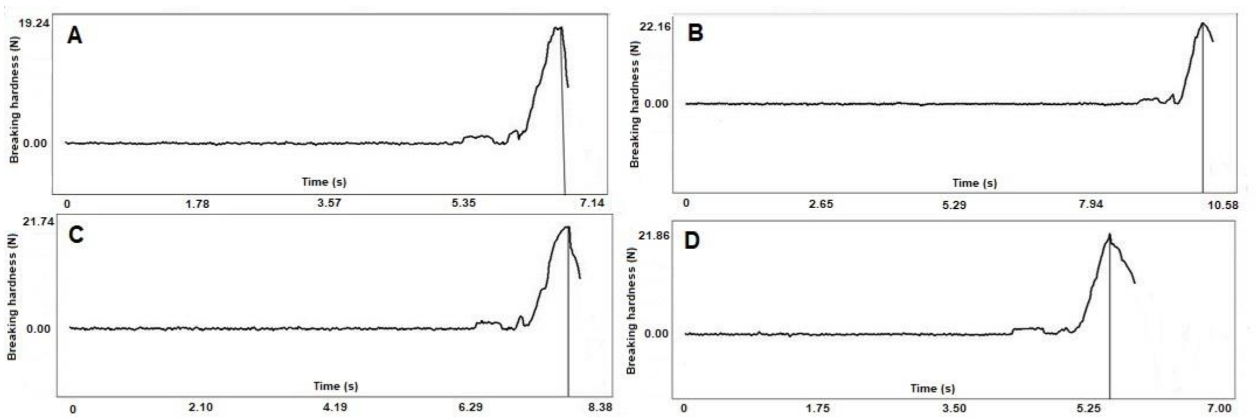

Figure 13. *Deformation curves of A. empty cellets, B. pellet molar ratio 2:1, C. 1:1, D. 1:2*

5.2.5. Dissolution test

Preparation of layered pellets leads to enhancing drug dissolution rate as it reduces disintegration time in addition to the presence of drug mixture on the pellets surface that makes it directly distributed and rapidly dissolved in dissolution medium.

Also, applying of high-shear granulator in this process generates a high mechanical force that help in the transformation of the physical form of drugs by reducing crystallinity index and proceeding amorphization and consequently increasing of drug release percent. On the other hand, this process was performed without the addition of a hydrophobic binder that may decrease the dissolution rate of the drug.

According to dissolution profile results (*Figure 14*), it found that amlodipine besylate release percent was slightly increased by 1.09, 1.07 and 1.1 times for layered pellets with molar ratios of 1:2, 1:1 and 2:1, respectively. As it mentioned in literature, amlodipine besylate is a Biopharmaceutics Classification System (BCS) Class 1 and rate-limiting step of dissolution process of this class in absorption, however, enhancing dissolution rate of this drug is related to increasing of surface area, destroying of it crystalline structure and the forming of supersaturated phases [74].

Moreover, hydrochlorothiazide release percent enhanced by 1.79, 2.45 and 2.59 times for pellets with molar ratio 1:2, 1:1 and 2:1, respectively even though it partially amorphized and this may due to the reducing crystallinity index that lead to form high energy state beside effect of amlodipine besylate (hydrophilic co-former) in improving wettability of layered pellets and enhancing dissolution of hydrochlorothiazide [75,76].

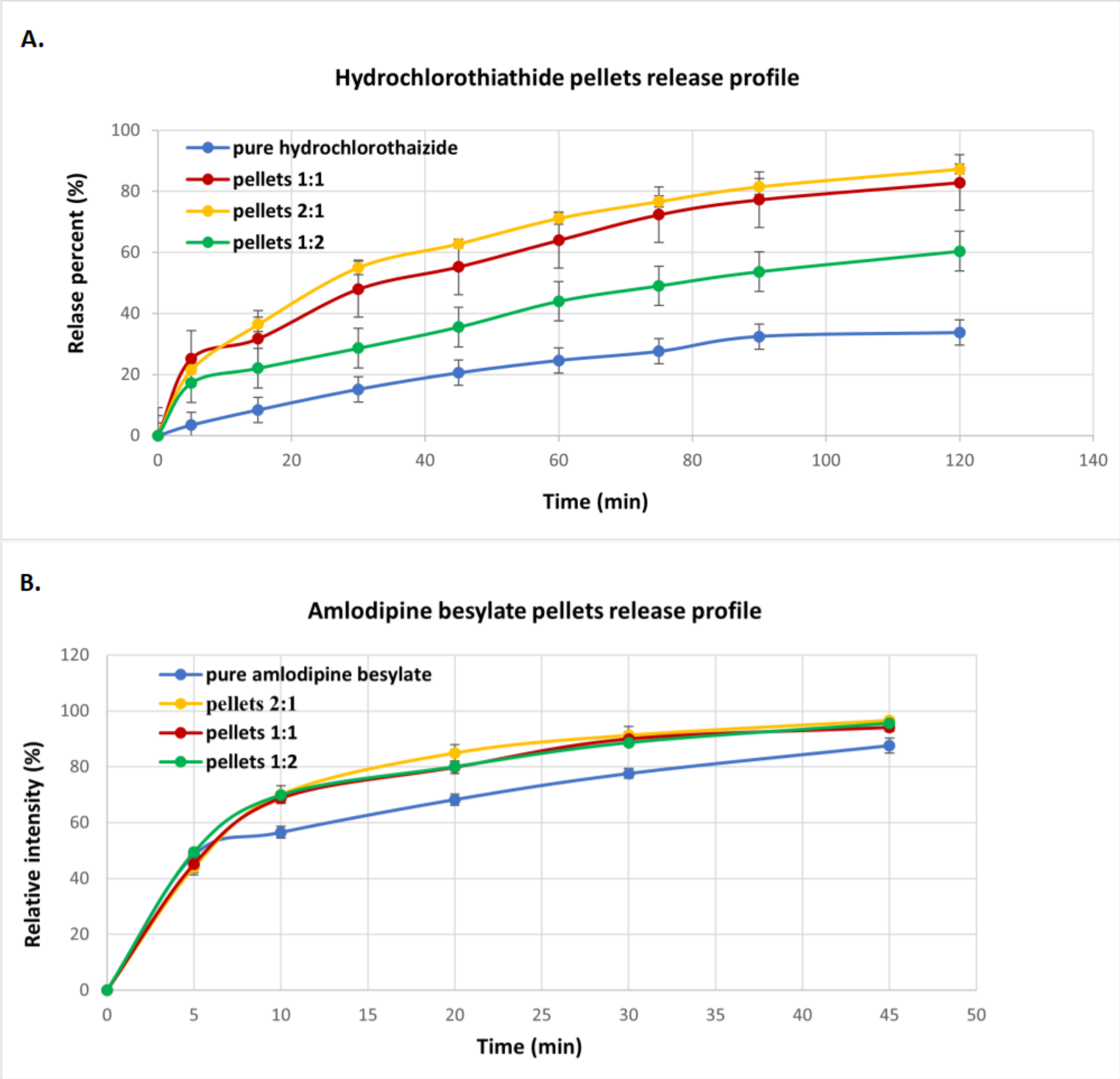

Figure 14. *Dissolution release profile for layered pellets containing A. amlodipine besylate and B. hydrochlorothiazide*

5.2.6. FTIR spectroscopy

FTIR was applied to determine the existence of molecular interactions between hydrochlorothiazide, amlodipine besylate and cellets. According to Figure 15, the pure amlodipine besylate had characteristic peaks at 3299 cm^{-1} that related to N–H stretching, specifically the secondary amine while the C–H stretching of the aromatic rings appeared at 3159 cm^{-1}. The C=C aromatic stretching, C=O stretching of amide and carbonyl group stretching were detected at 1617, 1676 and 1697 cm^{-1}, respectively [77].

The pure hydrochlorothiazide exhibited N–H stretching and aromatic C-H stretching at 3361 and 3170 cm^{-1} while a symmetric and symmetric stretching peaks of S=O stretching appeared at a range of 1300-1400 cm^{-1} and 1059 cm^{-1}. The aromatic group and C-N stretching were detected at 1608 and 1244 cm^{-1}, respectively [78].

Spectra of all layered pellets with different molar concentrations showed broadening of the primary sulphonamide and secondary amine of hydrochlorothiazide with merging with N-H stretching of amlodipine besylate at a range between 3300-3260 cm^{-1} that related to the destruction of the crystalline lattice of pure drugs [46].

Also, pellets with a 2:1 and 1:1 molar ratio spectra exhibited disappearance of the peak of a symmetric stretching of S=O of hydrochlorothiazide at 1059 cm^{-1} which may be related to hydrogen bond formation that changed the molecular environment and consequently enforced a symmetric stretching and reduced or shifted or weakened the symmetric peak [79].

Moreover, all other characteristic peaks of the drugs appeared without any shift and these results indicated that the application of a high-shear granulator influenced the molecular interaction between amlodipine besylate and hydrochlorothiazide.

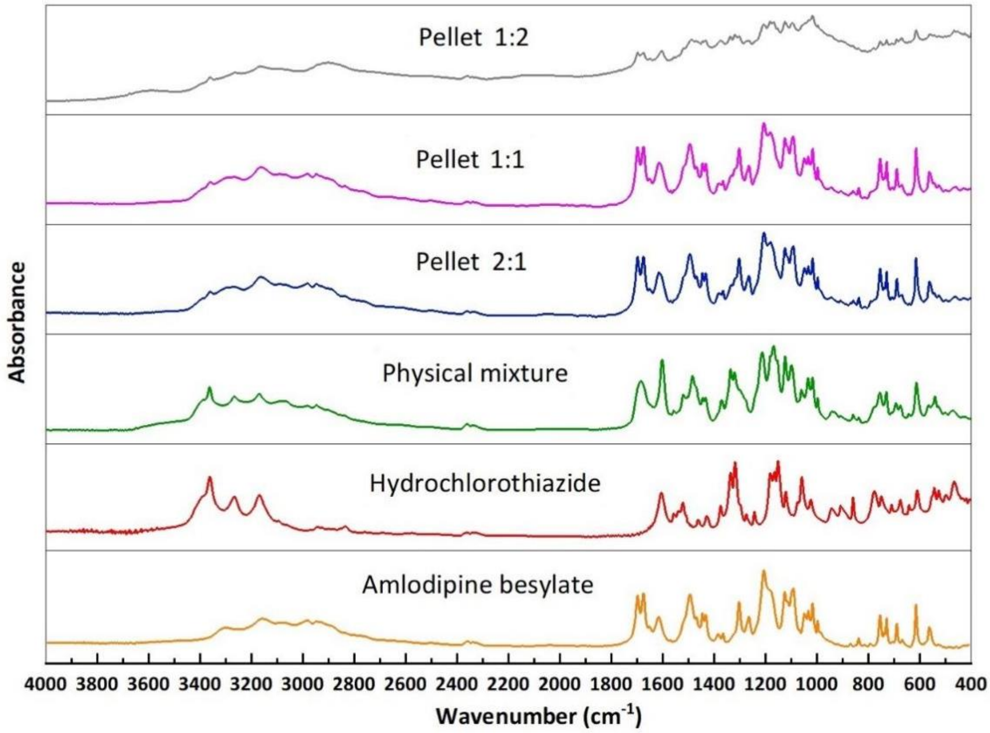

Figure 15. *FTIR of pure amlodipine besylate, hydrochlorothiazide and layered pellets.*

5.2.7. Stability studies

The XRPD diffractogram was used to estimate the physical transformation of drugs in layered pellets during the storage period of 1 month (*Figure 16*). It found that layered pellets at a molar ratio of 1:2 had the highest physical transformation and the lowest stability, which may be due to lower molecular binding in formulated pellets.

In contrast, layered pellets with molar concentration 1:1 and 2:1 exhibited a slight physical transformation and a higher stability (crystallinity index increased by 1 and 3% respectively) (*Table 16*) despite partial amorphization of both formulations.

Table 16. *Crystallinity index (%) after stability study*

Samples	Crystallinity index (%)
1:1 pellet	34.0
2:1 pellet	29.1
1:2 pellet	79.9

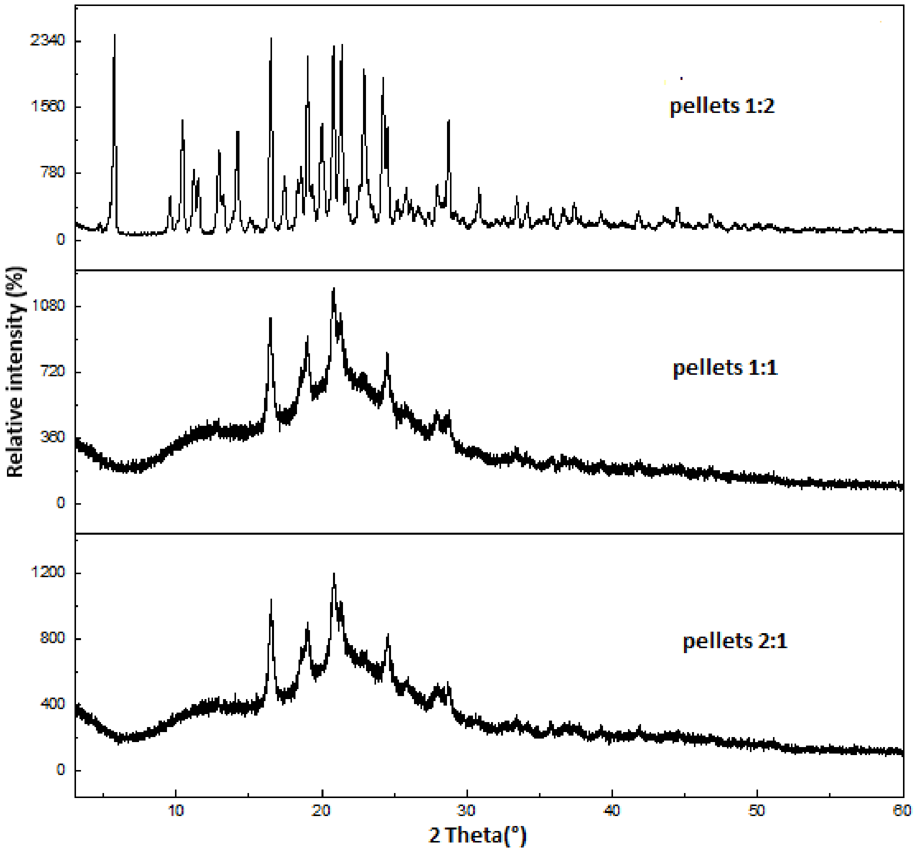

Figure 16. *XRPD diffractogram of stability study*

6. CONCLUSIONS

A single-step direct pelletization technique was performed by high-shear granulator with aid of the QbD tools, including fish bone diagram, risk estimation matrix and Pareto chart that contributed to determine CQAs with the highest risk values involving aspect ratio, yield percent and breaking hardness while process variables with the highest values involving impeller speed, binder amount, chopper speed, granulating liquid volume and liquid addition rate. Three different DoE, particularly screening design was used to provide primitive information about the effect of process variables on CQAs and the exclusion of liquid addition rate as it had a non-significant effect on any of the CQAs. Additionally, the process optimization and validation were carried out by central composite design and two-level full factorial design and it successfully constructed a design space with desired CQAs, including aspect ratio < 1.2 beside breaking hardness < 33 N and yield percent > 80%.

The optimized experiment was performed with the inclusion of hydrochlorothiazide and amlodipine besylate, which act as model drugs and it was found that there is no significant difference between aspect ratio and breaking hardness of pellets containing both drugs compared to plain pellets. However, the yield percent of pellets containing hydrochlorothiazide is significantly different ($p > 0.05$) from the plain pellets as it has a hydrophobic nature in addition to enhancing the dissolution profile that correlated to electrokinetic potential results. It considered as a promising technique for the preparation of different drugs as a multiparticulate system with extensive studies of scaling up.

On the other hand, a high-shear granulator was applied to perform single-step layering pelletization. Pellets containing MCC increased in size due to the loading of uniform layer of hydrochlorothiazide and amlodipine besylate combination on their surface based on micro-CT images. Moreover, both drugs physical form was modified due to change in its endothermic peaks and a decrease in crystallinity percent of the produced pellets with different molar ratios in DSC thermogram and XRPD diffractogram, respectively that also confirmed by an enhancement of their dissolution rate. Furthermore, the crystallinity percentage of pellets loaded with a high quantity of amlodipine besylate showed a lower decrease in crystallinity percentage compared to pellets loaded with low quantity of amlodipine after stability study.

Despite incomplete amorphization of the loaded drug mixture in layered pellets, this technique contributes to formulating pellets containing an anti-hypertensive combination with decreasing crystallinity index and improving drug release.

7. PRACTICAL USEFULNESS

- Production of pellets dosage forms based on predetermined CTPPs, CQAs and identified the highest risk process variables and responses that were checked through different DOE to provide in-depth information about direct pelletization techniques which can be applied in future studies.
- Estimation of applicable design space with desired CQAs ranges that contribute to reducing process cost and time.
- The optimized experiment of direct pelletization can be used to prepare different drugs as pellets dosage forms.
- Formulation of layered pellets containing an anti-hypertensive combination in a single step, focusing on the effect of different molar concentrations on the produced pellets.
- Appling high-shear granulator as a novel and economic tool that contributes to enhancing drugs amorphization and improving their release percent in a single step.

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10. DECLARATION OF OWN WORK

Name: Azza Asim Khalid Mahmoud

Title of the doctoral thesis: **APPLICATION OF HIGH-SHEAR GRANULATOR IN DIFFERENT PELLETIZATION TECHNIQUES**

I, **Azza Asim Khalid Mahmoud**, fully aware of my responsibility, declare that my doctoral (Ph.D.) dissertation prepared at the Doctoral School of Pharmaceutical Sciences of the University of Szeged is based on my own research results. During my research work, the publication of my results, and the writing of my dissertation, I acted according to the basic principles and recommendations laid down in the Code of Scientific Ethics of the Hungarian Academy of Sciences.

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Szeged, 06. 11. 2025

Azza Asim Khalid Mahmoud

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