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Formulation, characterization and evaluation of pellets loaded with amoxicillin

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ABSTRACT:

the formulation and characterization of amoxicillin tartaric acid loaded pellets showed a promising approach to enhance the dissolution profile and therapeutic efficacy of amoxicillin. Also, these prepared pellets could increase the palatability by masking the bitter drug into pellets and thus into the capsules. The outcomes of this research have the potential to contribute significantly to the development of advanced drug delivery systems for sustained release/controlled release, ultimately improving patient outcomes in the treatment of infection.

Introduction:

1. Pellets:

Pellets are defined as geometrically precise agglomerates, characterized as free-flowing, spherical or semi-spherical solid units with sizes ranging from 0.5 to 1.5 mm, designed for oral administration . Unlike granules and conventional agglomerates, pellets differ in shape, surface area, and possess a narrow particle size distribution (Lavanaya et al., 2011). They are utilized to control drug release from formulations, prevent dose dumping, and reduce variability in gastric emptying and intestinal transit time. Pellets also freely disperse within the gastrointestinal tract, helping to separate incompatible drugs, mask unpleasant tastes, and minimize irritant effects on the gastric mucosa (Kleinebudde et al., 2007). (Macho et al., 2021).

Benefits of Multi Unit Pellet System (MUPS) Compaction Over Traditional Modified-Release Tablets or Pellet-Filled Capsules

1.2.1.1 -Pharmacokinetic Benefits

Because to their tiny size and reduced risk of localized discomfort, micro-pellets found in MUPS move quickly yet uniformly from the stomach into the small intestine, improving medication absorption and increasing bioavailability. Rapid enteric coating dissolving is made possible by the uniform evacuation of micro pellets from the stomach into the small intestine (Kállai-Szabó et al., 2022). By using controlled-release formulations, drug release is more uniform, there is less chance of dosage dumping, and inter-subject differences are less likely.

1.2.1.2 - Pharmacodynamic Benefits: Due to pellets' quick and uniform stomach emptying and subsequent uniform drug dissolution in the digestive system as a result of their small size and higher surface area, uniform drug absorption is enhanced, leading to predictable and regulated pharmacological effect. As there are many more pellets in a MUPS dosage form than in a typical pellet-filled capsule, there is less chance of dose dumping (in the stomach) and partial drug release. This further reduces inter- and intra-subject variability in drug absorption and clinical response (Nalluri et al., 2010; Thies and Kleinebudde, 1999; Soh et al., 2013).

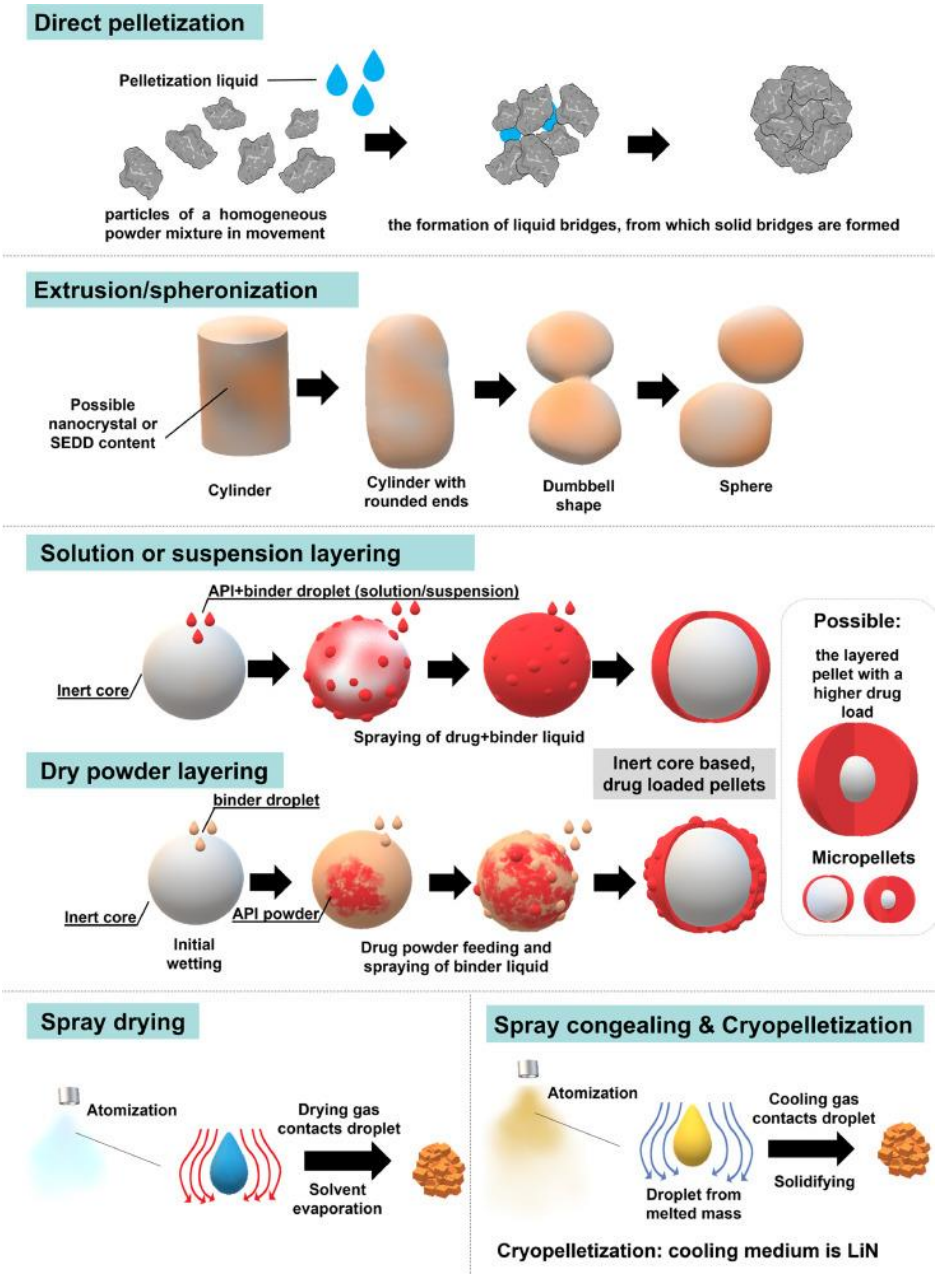


Figure: various methods of pellet formation

Physicochemical characterization and identification of amoxicillin

4.1.1 Physical appearance test:

Amoxicillin was classified based on several organoleptic qualities such as color, odor and appearance. The results were compared to the manufacturer's certificate of authenticity.

4.1.2 Melting point:

Amoxicillin's melting point was determined using the capillary technique

4.1.3 Fourier transform infrared spectral analysis

Amoxicillin's FTIR spectrum was detected by making potassium bromide pellets. Itraconazole powder was finely milled and combined with powdered potassium bromide before being pressed using a specified hydraulic compression (Liu, Vanderwyk et al. 2024).

5.3 UV standard-plot calibration curve

Methanol was chosen as the best solvent for amoxicillin spectrophotometric analysis.

Preparation of standard stock solution

A precisely weighed amount of 100 mg amoxicillin reference standard was put into a 100 ml volumetric flask, dissolved and diluted up to the mark with methanol to yield a stock solution with a strength of 1000µg/ml. Diluting 1 ml of stock solution to 10 ml with Methanol yielded a 100µg/ml working standard solution (Chavan, Bandgar et al. 2021).

Sample stock solution (100µg/ml) preparation

Twenty capsules were weighed and the mean weight was used to establish the amoxicillin content (label claim 500 mg amoxicillin per capsule). A weight of 100 mg amoxicillin powder was added to a 100 ml volumetric flask containing 50 ml methanol, and the combination was sonicated for 30 minutes before being diluted to 100 ml with methanol (1000 µg/ml). The solution was filtered, then 1 ml of the filtered solution was diluted tenfold to yield a concentration of 100µg/ml (Candiani, et al., 2022)

4.1.4 Determination of absorbance maxima (λ max)

The stock solution, i.e., 100 µg/ml was further diluted to get the concentrations (10,20,30,40 and 50 µg/ml) with methanol. The sample of prepared stock solution was subjected to UV-visible radiation between the wavelength range of 200-800 nm to determine the absorption maxima of the given sample. Each sample was determined thrice and absorbance readings were taken. The values are then expressed in mean values (Khurana, Agarwal et al. 2021) (Tumati and W/W 2024).

4.3 Preparation of pellets

The process of pellet formation proceeds in 4 Steps

4.3.1 Feeding the solid material into the extruder and melting or plasticizing it in a thermal carrier, usually a low melting point wax or polymers (starting from high molecular weight polymers to low molecular weight polymers), such as vinyl polymers, co- povidone, polyethylene glycol, acrylates, and cellulose derivatives. Diffusion (ethyl cellulose, carnauba wax) and erosion (HPMC) are the drug release methods

4.3.2 Extruder conveys mass, flows through the die, and shapes molten substance into uniform cylindrical segments.

4.3.3 Extrude spheronization at high temperatures to deform by softening and aid in the formation of uniform spheroids.

4.3.4 Solidifying spheroids to achieve the appropriate form, die exit, and downstream processing. The form of extruded items is determined by the endplate die linked to the end of the barrel

4.3.1 Preparation of mass:

For the present study, amoxicillin was mixed with tartaric acid, methyl cellulose (MCC), starch and sodium carboxymethylcellulose were mixed initially to form the mass. Three different batches were prepared to form the mass, as shown in table. The concenetrations of tartaric acid and MCC were varied to prepare the batches. The total batch size prepared was 5g for each batch (Huttner, et al., 2020).

Table - Different batches prepared using varied concentrations of citric acid and MCC.

S.no	Ingredients	F1	F2	F3
1	Amoxicillin	1g	1g	1g
2	Tartaric acid	0.50g	0.75g	1g
3	MCC	2.25g	2g	1.75g
4	Starch	0.75g	0.75g	0.75g
5	Carboxy methyl cellulose	0.5g	0.5g	0.5g
6	Total weight	5g	5g	5g

4.3.2 Extrusion of the mass

Each batch of the prepared mass (F1-F3) was then passed from the extruder, so as to obtain extrudes by applying a uniform pressure. On the basis of this, final batch of the prepared mass was then selected, and further spheronization process was performed on the selected batch. In F1 and F3 batches, the extrudes were not well formed and were slightly sticky, hence was not spheronized and rejected. The F2 batch exhibited the cylindrical extrudes.

4.3.3 Spheronization of the extrudes: The selected batch, F2, was then subjected to process of spheronization. The cylindrical extrudes obtained were then spheronized, so as to obtain the round spheres by applying heat and softening the extrudes.

4.4 EVALUATION OF PELLETS

4.4.1 Bulk density and tapped density

The specified amount of formulation is transferred to the measurement cylinder, and the cylinder volume is measured. The following formula is used to compute tapped density

Bulk density = weight of sample in g /volume occupied by the sample in Ml

A certain amount of the formulation is delivered to the measuring cylinder and physically tapped, either manually or with a mechanical instrument, until a consistent volume is attained.

Tapped density = Wt. of sample in g / Tapped volume in mL

It is easily determined by the USP density equipment. An automated tapper can be used to estimate the bulk density of pellets, while an air-comparison pycnometer or the solvent displacement technique can be used to determine the real density of pellets. The packing features of pellets or spherical seeds that produce greater bulk densities due to tiny intraparticle porosities are shown by bulk density. The amount of densification or compactness of pellets is indicated by true density.

4.4.2 The compressibility index of Carr: The compressibility index (C.I.) or Carr's index value of micro particles was calculated using the equation:
Carr's index = [Tapped density – Bulk density/Tapped density] X 100
A score less than 15% suggests a powder with good flow properties, whereas a value more than 25% indicates a powder with poor flow capabilities (Mohanthi, Ramya et al. 2022).

4.4.3 Haussner's coefficient : Hausner's micro particle ratio was calculated by comparing the tapped density to the bulk density using the following equation:

Hausner ratio = Tapped density/Bulk density

4.4.4 Angle of Repose : Solid flow qualities have been described using the angle of repose. Angle of repose is a property linked to interparticle friction or resistance to particle movement. This is the greatest possible angle between the surface of a pile of powder or grains and the horizontal plane. The fixed funnel and free-standing cone techniques use a funnel with its tip fastened at a set height, h, which was held 2 cm above graph paper on a level horizontal surface. The angle of repose may be calculated using the following equation, where r is the radius of the base of the conical pile:

$$\theta = \tan^{-1}(h/r)$$

Where, θ is the angle of repose, *h* is the height and *r* is the radius (Dhumal, Treffer et al. 2024).

4.4.5 Drug content: Drug content was determined in both the drug-containing core and the final functioning coated pellets. The drug content was calculated using a calibration curve.

4.4.7 Particle Size Distribution: The process of determining the size of pellets using Vernier callipers. The produced pellets were estimated using the sieving technique. Weight distribution is obtained directly from the sieving process. The sieves were stacked in a nest, coarsest at the top. A sample (5 gram) of dry pellets was deposited on the top filter and mechanically agitated. For a predetermined amount of time (10 minutes), the sieve set was fastened and shook. Each sieve's retained pellets were weighed. Pellets were frequently designated the mesh number of the screen through which they travelled or on which they were held. It was calculated using the Arithmetic mean of the two sieves (Chang, Yang et al. 2024).

$$\text{Mean particle size} = \Sigma XiFi / \Sigma Fi$$

Where, ΣXiFi = Weight size ; ΣFi = Percent weight retained.

4.4.8 Friability: Friability is a measure of the ability of a material to tolerate attrition during production, transportation, and storage. A friability of less than 0.8% is commonly acceptable for tablets, however this value may be greater for pellets because to the larger surface area/unit and consequent frictional force involvement. Pellet friability was tested in the same equipment with the equal amount of sample (10 g) and 200 total number of revolutions. the weights of the formulations were accurately recorded, and the friability ratios were calculated. The results were expressed in terms of the percentage of weight lost during the process.

4.9 Physicochemical Characterization of amoxicillin pellets

4.9.1 Particle size and shape distribution: Microscopic approaches are fundamental for studying size distribution. Pellets were evaluated for size and shape determination using optical microscopy with Led and camera.

4.9.2 Dissolution studies :

The dissolution investigations were conducted in two stages. Dissolution in acidic condition, 0.1N HCl, for 2 hours with volume 250 mL, USP apparatus I (Basket), and temperature 37±0.5°C, followed by dissolution in pH 5.5, simulated intestinal condition by 0.1N NaOH, for Another 2 hours with volume 250 mL, total of 500 mL in USP apparatus I (Basket), and temperature 37±0.5°C (Jakhar, Kaur et al. 2023).

The USP dissolving equipment 1 was used.at a temperature of in 250 mL of 0.1 N HCl (pH 1.1) solution 37°C±0.5°C. The disintegration apparatus's paddle speed was maintained at 75 revolutions per minute. Using 1-mL syringes, aliquots from dissolution vessels were collected after 10, 20, 30, 45, 60, 90, and 120 minutes and filtered through 0.45 mPTFE membrane filters pre-saturated with drug solution before being analysed using UV-spectrophotometer.

The pH of the dissolving media was then raised to 5.5 using 0.1 N NaOH to replicate the duodenal pH condition and to look for drug precipitation at higher pH . Because the drug particles that precipitated out after the pH shift to 5.5 had a propensity to settle at the bottom of the dissolving vessel when the rotation speed of the paddle was 75 RPM (Sardana, Khurana et al. 2019).

Result and Discussion

Physicochemical characterization and identification of amoxicillin

5.1.1 Physical appearance test:

Amoxicillin was classified based on several organoleptic qualities such as color, odor, and appearance. The results were compared to the manufacturer's certificate of authenticity.

5.1.2 Identification and 0characterization of amoxicillin

Table 8: Certificate of analysis of amoxicillin parameters

Parameter	Specifications as per COA	Observations
Physical state	Solid	Solid
Color	White	White
Odor	Odor less	Odor less

5.2.1 Melting point analysis

The observed experimental melting point by capillary method complies with the reported melting point as shown in table

Table : Certificate of analysis of amoxicillin parameter melting range.

Parameter	Specification as per COA	Observation
Melting range	194°C	191-196°C

5.3 UV Standard plot of Itraconazole:

The results of UV standard plot are shown in table . Five different concentrations of amoxicillin were taken (10,20,30,40 and 50 mcg/ml). The readings for each concentration were taken in triplicate and the mean absorbance was calculated. The absorbance readings were plotted for each concentration and regression line was obtained by setting zero intercept .

Table : Standard plot of amoxicillin

S. No.	Concenetration (mcg/ml)	Mean Absorbance
1.	10	0.241
2.	20	0.406
3.	30	0.588
4.	40	0.793
5.	50	0.946

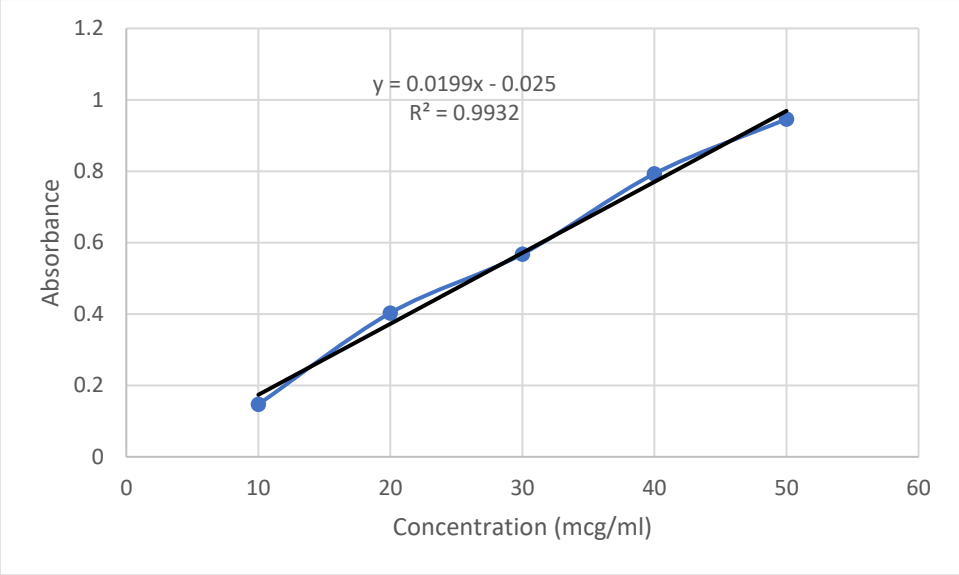


Figure : Calibration curve of amoxicillin by UV spectroscopy

FTIR Identification and Analysis –

Fourier transformed infrared spectroscopy (FTIR) studies were conducted. Samples were placed on the sample holder and the distance between the sample holder screw was adjusted to obtain cumulative results of 64 scans after background correction. The spectra were recorded over the range of 4000 to 400 cm−1 at the resolution of 4 cm−1

Data analysis, interpretation and comparison:
Infrared (IR) spectroscopy is a key analytical technique used to characterize functional groups in a molecule. The IR spectrum of **amoxicillin**, a β-lactam antibiotic, exhibits characteristic absorption bands corresponding to its functional groups, including the β-lactam ring, amide, carboxyl, hydroxyl, and aromatic groups.

Key Functional Groups and Corresponding IR Absorption Peaks

1. **Hydroxyl (-OH) Stretch (Alcohol & Carboxyl)**
    - Broad absorption around 3300–3500 cm^{-1}.
    - This corresponds to the presence of hydroxyl (-OH) groups in amoxicillin, specifically from the carboxyl (-COOH) and phenolic (-OH) groups.
2. **Amide (-NH) Stretch**
    - Peaks at 3100–3500 cm^{-1}.
    - This corresponds to the secondary amide (-NH) stretching from the amide functionality present in the β-lactam ring.
3. **C-H Stretching (Aliphatic & Aromatic)**
    - Aliphatic C-H stretching: Around 2850–2950 cm^{-1}.
    - Aromatic C-H stretching: Around 3000–3100 cm^{-1}.
    - These peaks are attributed to the presence of alkyl (-CH_3, -CH_2-) and aromatic (-C_6H_6) groups in amoxicillin.
4. **β-Lactam Carbonyl (C=O) Stretching**
    - Strong peak at ~1760–1790 cm^{-1}.
    - This is a critical peak for identifying the β-lactam ring, as it is shifted to a higher frequency due to ring

strain.

5. **Amide Carbonyl (C=O) Stretching**
   - A strong absorption at ~1660–1700 cm^{-1}.
   - This corresponds to the amide carbonyl (C=O) stretch, distinguishing it from the β-lactam C=O.
6. **Carboxyl (C=O) Stretching**
   - A strong peak at ~1720 cm^{-1}.
   - This peak represents the carboxyl (-COOH) functional group.
7. **C-N Stretching**
   - Peaks around 1200–1350 cm^{-1}.
   - Associated with the C-N stretching vibrations in the amide and β-lactam ring.
8. **C-O Stretching (Ester & Carboxyl)**
   - Observed around 1100–1300 cm^{-1}.
   - This results from the stretching of the C-O bond present in the carboxyl (-COOH) and other oxygen-containing functional groups.
9. **Aromatic C=C Stretching**
   - Peaks around 1450–1600 cm^{-1}.
   - These represent C=C stretching in the benzene ring of amoxicillin.
10. **β-Lactam Ring Deformation (Fingerprint Region)**
    - Peaks in the 600–900 cm^{-1} region.
    - These correspond to skeletal vibrations associated with the β-lactam ring structure.

The β-lactam C=O stretch (~1760 cm^{-1}), amide C=O (~1660–1700 cm^{-1}), and carboxyl C=O (~1720 cm^{-1}) are the most significant peaks in amoxicillin's IR spectrum. The presence of hydroxyl (-OH), amine (-NH), and aromatic C=C peaks further confirms the functional groups characteristic of amoxicillin.

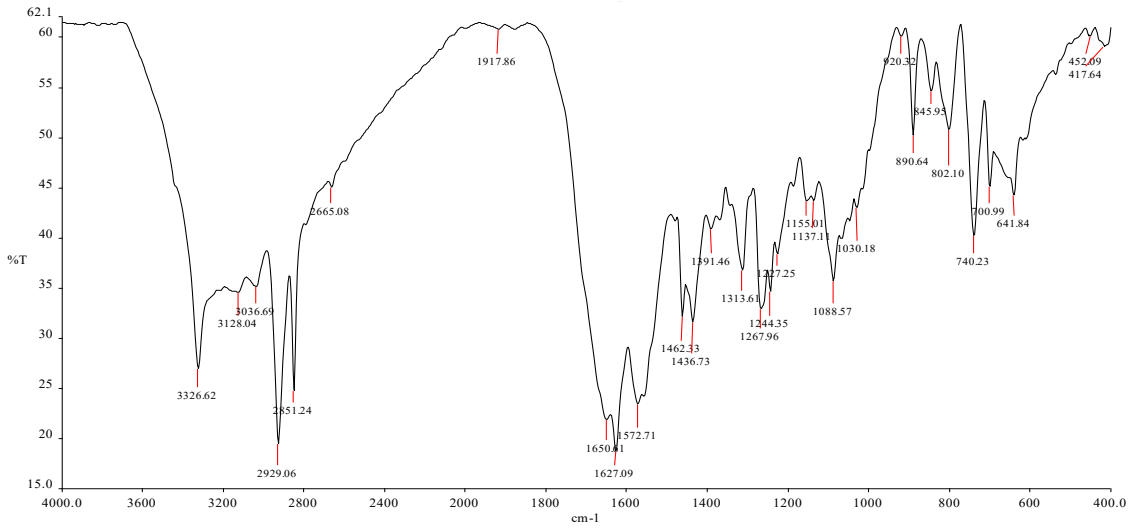


Fig 21. FTIR of ITZ reference standard

pH-solubility Study:

The solubility of amoxicillin as a function of pH was evaluated in 10% 0.1 N HCl solution by adding tartaric acid to aqueous solutions of amoxicillin and pH was determined using the apparatus pH meter. However, it was discovered that the pH remained virtually constant. After the addition of particular levels of acids, the substance remains unaltered. As a result, the approach was updated to determine pH and acid concentrations affect solubility. Solutions with known concentrations of tartaric acid, were prepared first based on their solubility in water, then excess amounts of amoxicillin were added to the acid solutions, the suspensions were shaken at 25±1°C for predetermined periods of time using vortex mixture, and the pH values were measured. (Parmentier, Tan et al. 2017).

Table 11. pH solubility study of amoxicillin with different conc of tartaric acid

S.no	Name of salt	Conc.ug/ml	pH
1	tartaric acid	0.50g/ml	2.41
2	tartaric acid	0.75g/ml	1.64
3	tartaric acid	1.0g/ml	2.75

Selection of the batch

Out of the three batches of the mass prepared, with different concentrations of tartaric acid, F2 (having 0.75g of citric acid), was finalised, the extrudes made from these were cylindrical, non-sticky and uniform. F1 batch was slightly hard and extrudes could not be formed easily. The extrudes of the F3 batch were slightly sticky as compared to that of F2. So therefore, F2 batch was finalised and the extrudes were formed. These extrudes were then subjected to the process of spheronization. Figure shows the spheronized product thus obtained.

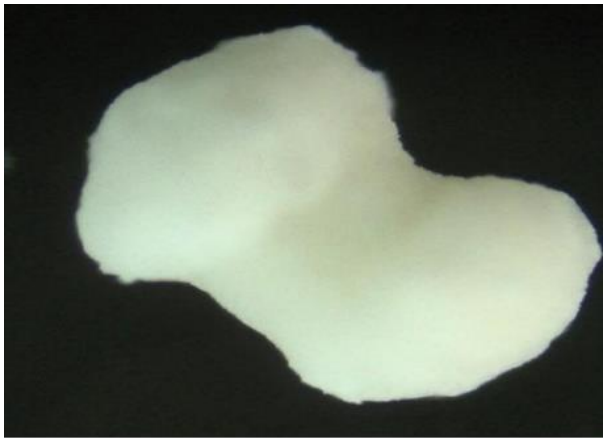
Fig.12. shows the spheronized product

5.7 Optimization of pellet -Extruder spherionzer

Result :

Table 15 : Tartaric Formulation -pictures has been taken on different intervals of time during spherionizing process for total of 120 seconds.

Time (sec)	Microscope with LED and camera	Remarks

30		Longitudnal cylindrical
60		Size reduced but still longitudnal cylindrical
90		Dumbell shaped

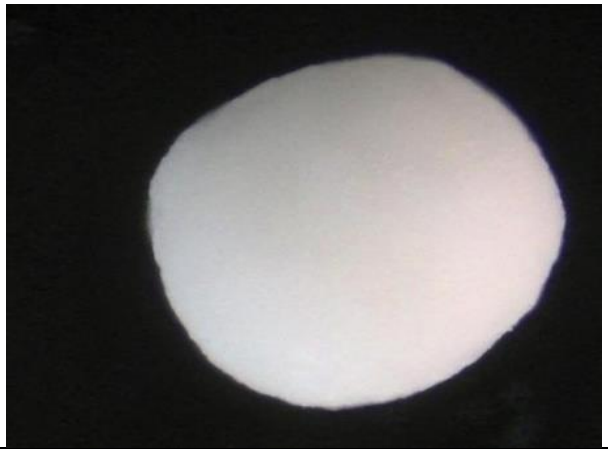
120		Spherical

Fig. Final pellets of amoxicillin with tartaric acid.

5.8 Characterization of the prepared pellets:

5.8.1 Bulk Density and Tapped Density: The bulk density and tapped density are the necessary parameters for determining the flow properties of the given material. Hence it becomes essential part to estimate the bulk and tapped densities for the prepared pellets. The results are given in the following table.

Table: Results of bulk density and tapped density

	Observed Bulk Density (g/ml)	Mean Bulk density (g/ml)	Observed Tapped density (g/ml)	Mean Tapped density (g/ml)
Pellets of itraconazole	0.67	0.62	0.73	0.67
	0.61		0.67	
	0.59		0.63	

5.8.2 Carr's Index: Carr's index is an indirect measure of the interparticulate forces within the particles and hence their flow ability. Both of them were calculated using the bulk and tap densities of the pellets batch and hence the Carr's index was calculated.

5.8.3 Haussner's ratio: the ratio of the tapped and the bulk density gives the Haussner's Ratio, which is an indirect measure for the ability of the particles to make a good flow. Ideally, the 1-1.11 ratio, signifies excellent flowability. The results observed are shown in the table. As the pellets had the good potential to flow.

	Carr's Index	Mean Carr's Index	Haussner's Ratio	Mean Haussner's Ratio
Pellets of	8.22	7.88	1.08	1.08

itraconazole	8.9		1.09	
	6.35		1.07	

5.8.4 Angle of repose: The flow properties of developed pellets was ranged in excellent micromeritic properties as evident from angle of repose test. The mean value (n=3) of angle of repose for developed pellets was observed to be 20° (less than 25°) which indicates the pellets fall under excellent category of powder flow. The observed values also conforms to the findings of VR Sinha et al. (2005) who reported the angle of repose value less than 25° and suggested good flow potential for developed pellets.

5.8.5 Particle size distribution: The particle size of pellets has an impact over rate of dissolution and uniformity in drug content. Therefore, uniformity in size distribution is important to define rate of dissolution and uniformity in drug content. From the results, it was observed that the pellet size was found to be 1658 ± 227 µ (Kállai-Szabó, Farkas et al. 2024) .

5.8.6 Friability: The results for the friability-i.e., the percentage loss in weight during the friability testing-are presented in Table . If these results are compared to those which can be found in the literature and which were obtained by comparable methods, the pellets seem to be strong enough to withstand rough handling. In literature, it is reported that pellets ranging in size around 600 µ showed less percentage weight loss i.e. between less than 1.8% as compared to the pellets with size range around 300 µ i.e. between 0.3 to 2.7%. Hence, concluding that pellets larger in size were having strong mechanical strength as compared to smaller size pellets. In our results the pellets with size range of 1658 ± 227 µ showed approximately 0.48% percentage weight loss and thereby comparing with literature reports the developed pellets seem to have strong physical and mechanical strength

SNo	**Sample**	**Angle of Repose**	**Particle size**	**Friability**
		Mean ± SD		
1	F1	20° ± 1	1658 ± 227 µ	0.48 % ± 0.11

5.8 Dissolution Study:

The amoxicillin and tartaric acid and its pellets showed very high dissolving rates when powdered and sieved (# 40 mesh). In the current study, pellets were employed for dissolving since the final dose form of the drug was effective.

The binary combination of amoxicillin with tartaric acid (20% drug load) was combined with a super disintegrant (10% w/w) and compressed into pellets. 100 mg amoxicillin (total capsule weight=500 g). The capsules started dissolving in 250 mL of 0.1 N HCL. (pH 1.5) for 125 minutes at 75 RPM and 37°C, and then the vol. of dissolution media was made up to 500mL with pH adjustment to 5.5 using 0.1N NaOH.

Absorbance of drug take place from 0-120 min in acidic medium and after maintaining pH 5.5 as per duodenal intestinal pH. D rug dissolution was subsequently decreased after 150 mins in the prepared formulations, as clearly evident from the graph. (Chang, Yang et al. 2024).

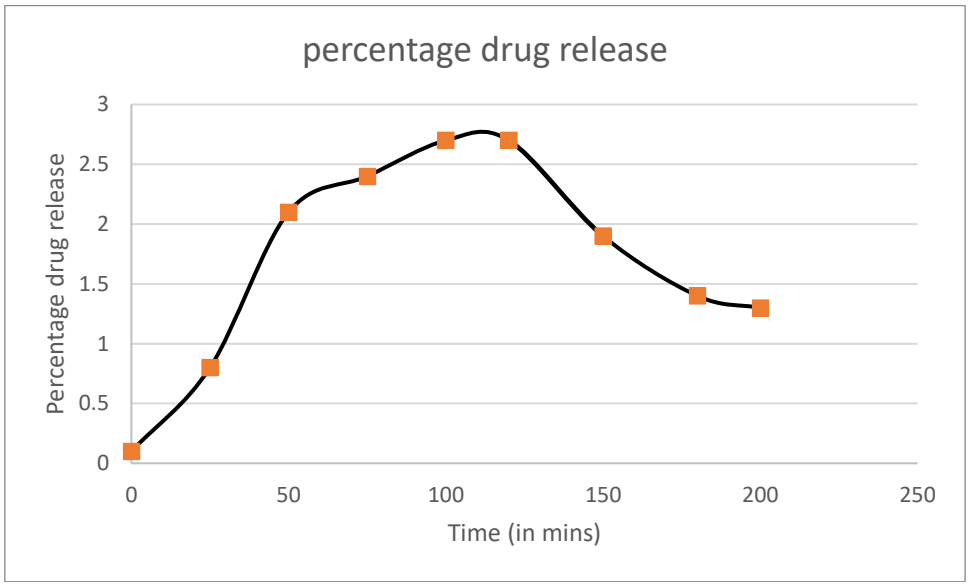


Fig. Percentage drug release from dissolution studies

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