

ORIGINAL ARTICLE**Preparation and Evaluation of Immunosuppressant dispersible tablet**

¹Pratik S. Kadam, ²Prashant L. Pingale, ³Dattatraya M. Shinkar, ⁴Sahebrao S. Boraste, ⁵Smita P. Kakad, ⁶Sunil V. Amrutkar

^{1,2,3,4}Department of Pharmaceutics, Gokhale Education Society's Sir Dr. M.S. Gosavi College of Pharmaceutical Education and Research, Nashik-422005 (M.S.), India

⁵Department of Pharmaceutics, MET Institute of Pharmacy, Nashik-422003 (M.S.), India

⁶Department of Pharmaceutical Chemistry, Gokhale Education Society's Sir Dr. M.S. Gosavi College of Pharmaceutical Education and Research, Nashik-422005 (M.S.), India

ABSTRACT

The preparation and evaluation of an immunosuppressant dispersible tablet using the top spray granulation method is described in this study. Immunosuppressants play a crucial role in preventing the rejection of transplanted organs and are widely used in organ transplantation and autoimmune disease treatments. However, the development of patient-friendly formulations, such as dispersible tablets, is necessary to enhance medication adherence, especially in cases where patients have difficulty swallowing conventional tablets or capsules. In this research, a top spray granulation technique was utilized to produce dispersible tablets that incorporated an immunosuppressant drug. The granulation process involved the formation of granules by spraying a binder solution onto the drug and excipient mixture. The resulting granules were then compressed into tablets. Various formulation parameters were investigated, including binder concentration, granulation time, and compression force, to optimize the dispersible tablet formulation. The dispersible tablets underwent a thorough assessment to determine their quality characteristics. The evaluation encompassed various tests such as weight variation, hardness, friability, DT and drug content uniformity. Moreover, the tablets were analyzed for their drug release behavior through in vitro dissolution studies. The evaluation results were compared with reference criteria to ensure compliance with regulatory standards.

Keywords: Miconazole nitrate, Mucoadhesive, Buccal patch, Sustained Release, Oral Candidiasis

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INTRODUCTION

In the field of medical science, immunosuppressant drugs play a vital role in preventing organ rejection following transplantation and treating various autoimmune diseases. However, the administration of immunosuppressants often poses challenges, especially in patients who face challenge in engulfing conventional tablets [1]. To address this issue, the development of dispersible tablets using the top spray granulation method has gained significant attention. This method offers a promising solution for improving drug delivery, particularly when utilizing mTOR inhibitor drugs.

mTOR (mammalian target of rapamycin) inhibitors have revolutionized the field of immunosuppression, demonstrating superior efficacy and safety profiles compared to traditional immunosuppressive agents. These inhibitors target the mTOR pathway, which regulates crucial cellular processes, including cell growth, proliferation, and survival. By inhibiting mTOR, these drugs effectively suppress the immune response and prevent organ rejection [2]. However, the formulation of mTOR inhibitor drugs into dispersible tablets presents unique challenges due to their physicochemical properties and stability requirements [3].

The top spray granulation method has emerged as a popular technique for formulating dispersible tablets. This process involves spraying a binder solution onto the powder bed, leading to granule formation. The resulting granules exhibit improved flow properties and compressibility, facilitating the

manufacturing of tablets with desirable characteristics such as rapid disintegration and enhanced dissolution rates. Additionally, the top spray granulation method allows for the incorporation of excipients that enhance tablet stability, bioavailability, and patient compliance [4,5].

The development and evaluation of immunosuppressant dispersible tablets using the top spray granulation method offer several advantages. Firstly, these tablets provide an alternative dosage form for patients who have difficulty swallowing conventional tablets, including pediatric, geriatric, and dysphagic individuals. Secondly, the improved drug delivery and disintegration properties of dispersible tablets ensure efficient drug absorption and therapeutic outcomes. Moreover, the use of mTOR inhibitors as the active pharmaceutical ingredient (API) in these tablets further enhances their clinical significance [7].

This research aims to explore the preparation and evaluation of immunosuppressant dispersible tablets utilizing the top spray granulation method, with a specific focus on mTOR inhibitor drugs. The investigation will encompass various aspects, including the selection of suitable excipients, optimization of formulation variables, evaluation of physicochemical properties, and assessment of in vitro and in vivo performance. The goal is to develop a stable and effective dosage form that can improve patient compliance, enhance therapeutic outcomes, and pave the way for personalized immunosuppressive therapy. This research seeks to enhance our comprehension of the top spray granulation technique used to formulate dispersible tablets of immunosuppressants. By doing so, it aims to make significant contributions to the pharmaceutical sciences and offer valuable knowledge for the advancement of innovative drug delivery systems in the field of immunosuppression.

MATERIAL AND METHODS

Materials

Along with the API the preparation of the dispersible formulation includes various excipients with specific functions. The binder agents used are Hypromellose (Methocel E3 Premium LV) and Hypromellose (Methocel E5 Premium LV), which help in holding the tablet ingredients together. For disintegration purposes, Croscopovidone (Kollidon CL-F) is employed. To enhance the flow properties of the formulation, Colloidal silicon dioxide (Aerosil 200 Pharma) is used as a glidant. Mannitol (Pearlitol SD 100) and Microcrystalline Cellulose (Avicel PH 200 LM) are added as diluents to increase the bulk of the tablet. Lastly, for lubrication, Magnesium Stearate (Ligamed MF-3-V) is incorporated, ensuring smooth tablet manufacturing, and preventing sticking to the machinery. Additionally, Butylated Hydroxy Toluene is included as an antioxidant to preserve the stability of the formulation during storage. These excipients, with their specific functions, collectively contribute to the successful preparation of the dispersible tablets.

Method of preparation of dispersible tablet:

The dispersible tablet was prepared using the solid dispersion (Top spray granulation) method. In this technique, a poorly water-soluble drug was dispersed within a solid matrix of a chosen carrier material, typically a polymer. The drug and carrier were mixed, and then granulated using the top spray method, where a binder solution was sprayed onto the powder bed in a fluidized bed system. The damp solid dispersion was dried to remove the solvent. The resulting material was compressed into dispersible tablets designed to quickly disintegrate in water, enhancing drug dissolution and bioavailability [7,8].

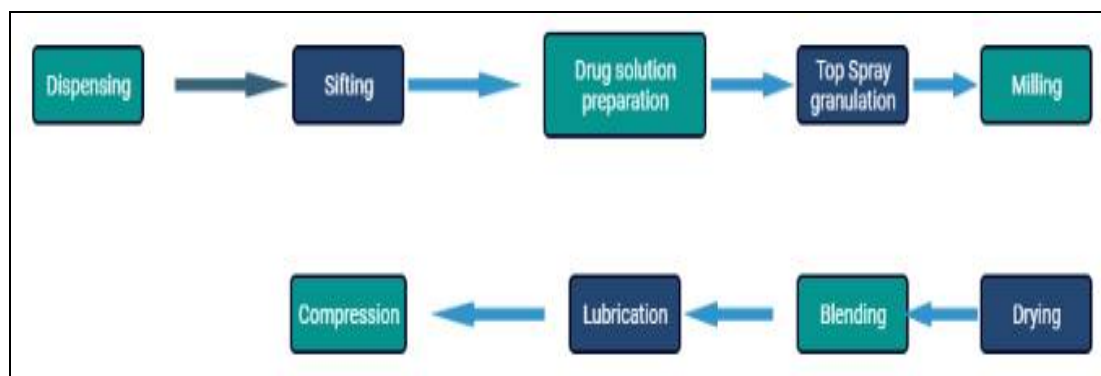


Figure 1: Steps involved in the preparation of dispersible tablets

Drug excipient compatibility study [9]:

The compatibility of a drug substance with various excipients used in the formulation was studied under accelerated conditions of temperature and humidity(40°C/75%RH). Binary mixtures of the drug

substance and excipients were analyzed using HPLC in the solid state, with a specific ratio. The samples were stored in open amber colored glass vials under initial conditions for one month. Common excipients acting as diluent, binder, disintegrant, glidant, and lubricant were assessed. The appearance, assay, and total degradation products of each combination were monitored before and after the one-month storage period. These results provide valuable insights into the stability and suitability of the excipients for the formulation of the drug.

Formulation of dispersible tablet:

Formulation development aims to create a reliable product that is safe, effective, and stable. The selection of a prototype formulation is based on its dissolution and disintegration profile, which measures how quickly and completely the drug dissolves in the body. For low-dose formulations, wet granulation was chosen as the manufacturing process. Specifically, top spray granulation was preferred due to its ease of handling for low-dose potent molecules and its ability to achieve a uniform distribution of the drug substance in the final product. This approach ensures that the formulation is consistent, reliable, and meets the desired therapeutic outcomes.

Table 1: Formulation design of dispersible tablet batch no. 1 to 6

Batch No.		1	2	3	4	5	6
Sr. No	Ingredients	mg/tablet					
Drug dispersion materials							
1	Drug substance (0.2% BHT)	5	5	5	5	5	5
2	Butylated hydroxytoluene (BHT)	0.1	0.1	0.1	0.1	0.1	0.1
3	Hypromellose (Methocel E3 Premium LV)	41.25	45	48.75	45	45	45
4	Hypromellose (Methocel E5 Premium LV)	27.5	30	32.5	30	30	30
5	Colloidal silicon dioxide (Aerosil 200 Pharma)	6.25	6.25	6.25	6.25	6.25	6.25
6	Acetone	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
7	Ethanol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Intra granular materials							
8	Mannitol (Pearlitol SD 100)	270	270	270	270	270	270
Extra- granular materials							
9	Microcrystalline Cellulose (Avicel PH 200 LM)	212.4	206.15	199.9	193.65	199.9	190.525
10	Crospovidone (Kollidon CL- F)	50	50	50	62.500	75	62.5
11	Colloidal silicon dioxide (Aerosil 200 Pharma)	6.25	6.25	6.25	6.25	6.25	6.25
12	Magnesium Stearate (Ligamed MF – 3 – V)	6.25	6.25	6.25	6.25	6.25	9.375
Total weight of core tablets		625	625	625	625	625	625

A) Pre-formulation Parameters:

Bulk density and tapped density:

Without compacting the sample, place it into the measuring cylinder and then read the unsettled apparent volume, V_o m. Use the same powder sample to perform 500 and 1250 taps, and then read the associated volumes V_o and is to the closest graded unit. V_t is the tapped volume (VD) if the difference between the two measurements is less than or equal to 1 ml. Repeat until the difference between subsequent measurements is less than or equal to 1 mL if the difference between the two is greater than that amount. This will let you to determine the tapped volume (VD) [10]. Calculate the untapped density (bulk density) and tapped density as follows:

D_b = Mass of powder/Volume of powder

D_t = Mass of powder/Tapped volume of powder

Compressibility index:

It is determined through the assessment of bulk density and tapped density values. The index is computed using the following formula: Carr's index equals the difference between tapped density and bulk density, divided by tapped density, multiplied by 100.

Hausner's ratio:

This ratio indicates the flow properties of the powder or blend and is the ratio of tapped density to bulk density: Hausner's ratio = TD/BD [11].

Angle of repose:

The angle of repose for the granules was measured using the funnel technique. A predetermined amount of granules was placed into the funnel, and the height of the funnel was adjusted to ensure that its tip made minimal contact with the granule pile. The granules were then allowed to flow freely through the funnel and onto a surface [12]. By measuring the diameter of the resulting cone of granules, the angle of repose was calculated using a specific equation.

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

Where, θ = Angle of repose, h = Height of heap of powder, r = Radius of heap of powder

Particle size distribution of powder:

The variety of particle sizes contained in a powder or particulate substance is referred to as the particle size distribution. It is a crucial quality that influences how powders behave and operate in a variety of settings, including the manufacturing of drugs, cosmetics, food, and materials. The analysis of particle size distribution frequently involves the use of methods like sedimentation, microscopy, or sieving. Among those techniques we had used sieving method for the determination of particle size.

B) Post-compression parameters:**General Appearance:**

Gaining consumer approval depends heavily on the general appearance, visual identity, and "elegance" of a tablet. The size, shape, color, aroma, taste, texture of the tablet's surface, physical faults, consistency, and legibility of any identifying markings are all considered.

Tablet thickness: Tablet thickness is a significant aspect when counting with filling equipment and matching appearance. Ten pills were measured for thickness using a Vernier caliper.

Hardness test: The tablet's hardness (tablet breaking strength) is defined as the amount of force required to break the tablet in a diametric compression test. It was measured using mechanical hardness tester. It's written as kg/cm.

Weight variation test [13]

Weight variation was checked by selecting 20 pills at random from the batch and weighing each one separately. Table shows the weight variation specification according to I.P.

Friability: This test evaluates the ability of tablets to withstand abrasion during packing, handling, and transportation. 6.5gm weighed tablets were taken and put in a friability apparatus, and the effects of abrasion and impact were examined by dropping the tablets six inches apart over the course of 100 rotations of a spinning plastic chamber moving at 25 revolutions per minute [14].

The tablets were then weighed again. Following that, the percentage for friability was determined using the following formula:

$$\text{Friability} = [\text{Initial Weight} - \text{Final weight}] / \text{Initial weight} \times 100$$

Disintegration time: The disintegration time of tablet was done through Disintegration test apparatus. The tablet was dispersed in the apparatus at room temperature and disintegration time in minutes was noted [15].

In vitro drug release: The *in-vitro* dissolving experiments were conducted using the USP-II (paddle) dissolve device at a speed of 75 rpm. Phosphate buffer with a pH of 6.8 is stored in dissolution medium at 37.5°C. 5 ml was taken out and replaced with the same amount of fresh medium at regular intervals. The withheld samples were diluted with pH 6.8, filtered, and analyzed at 210 nm on a UV spectrophotometer using pH 6.8 as a blank. The cumulative drug release percentage was calculated [16].

Content uniformity of tablet: Tablets are frequently created in big batches during the pharmaceutical production process. A uniform mixture of materials, including APIs and excipients, is created during the production process. The tablet press is then used to compress this mixture into tablets. It is essential that each tablet contains the appropriate amount of the active ingredient and that the active component is distributed uniformly throughout the tablet to provide the desired therapeutic effect [17].

The amount of the active ingredient contained in each tablet is measured using testing samples from a batch of tablets to assess content consistency. High-performance liquid chromatography (HPLC), which can precisely determine the quantity of the active substance, is frequently used in this testing.

DSC Study:

Glass transition temperature has been determined using DSC analysis for drug substance batches and results range of 85-91 degree Celsius. The DSC was carried out using Mettler Toledo DSC 822e module instrument. The sample were heated from 30°C up to 250 degree C under nitrogen atmosphere. The DSC analysis have been performed for API and blend of formulated batch [18].

Stability test: The dispersible tablets are packaged appropriately and kept under the following conditions for the duration of the accelerated stability study, as specified by the ICH guideline: 40±2°C and Relative Humidity of 75%–5%.

After a predetermined amount of time, the tablets are removed and their physical characteristics (including hardness, brittleness, dissolution, and other visual flaws) and drug content are examined. To evaluate the kinetics of deterioration, the acquired data is fitted into a first order equation.

RESULT AND DISCUSSION

Solubility: The solubility of API was determined in distilled water, 4.5 pH acetate buffer, 6.8 pH phosphate buffer, 7.5 pH phosphate buffer, 0.1N HCL, Ethanol, chloroform, methanol. Solubility studies in those media are shown in table 2. The result shows maximum solubility in ethanol, chloroform, and methanol.

Table 2 : Solubility study of API

Solvent	Solubility range	Solubility
Distilled water	0.1 mg/ml	Practically insoluble
4.5 pH acetate buffer	0.5 mg/ml	Very Slightly soluble
7.5 pH phosphate buffer	0.5 mg/ml	Very Slightly soluble
Ethanol	3 mg/ml	Moderately soluble
Chloroform	10 mg/ml	Sparingly soluble
Methanol	3 mg/ml	Moderately soluble

Melting point: Melting point of drug was determined by capillary method [19]. The melting point was found to be 85-90° Celsius.

UV Spectroscopy: When the laboratory standard and the sample dissolved in methanol are analyzed using UV spectroscopy in the wavelength range of 200-400 nm, both exhibit a concentration of 10 parts per million (ppm). The UV spectrum reveals a distinct peak (UV maxima) at approximately 277 nm, and the absorbance at this wavelength is measured to be approximately 0.500.

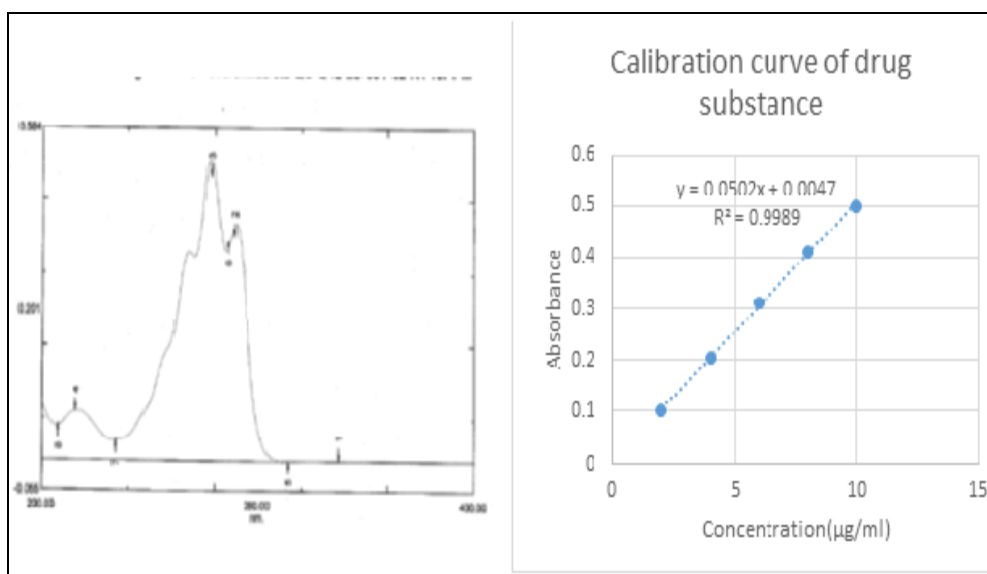


Figure 2: Calibration curve of API and Spectrophotometric Graph

Structural Elucidation: The structural elucidation was performed from the FTIR spectrum of the drug [20]. The key functional moieties in the structures of drugs are shown in the spectrums. The table indicates the key functional groups observed in the FTIR plots of the drugs.

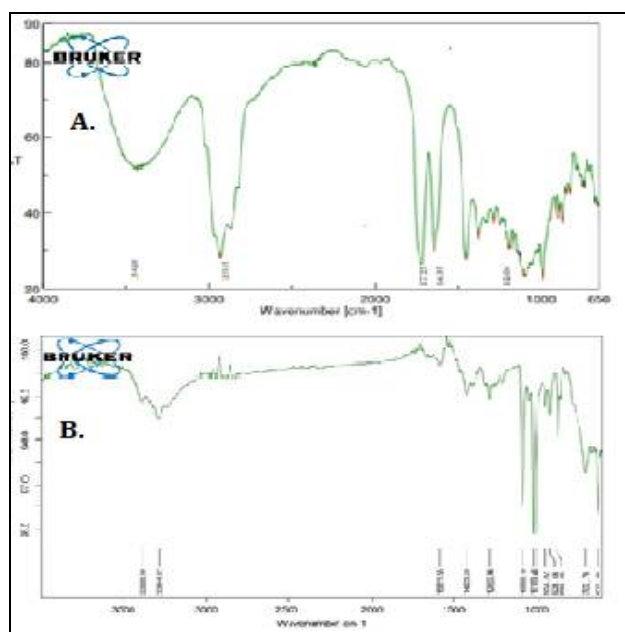


Figure 3: A. FT-IR spectra of Miconazole nitrate drug and B. FT-IR spectra of Formulation

The FTIR data suggests that both the API and the excipients contain functional groups such as hydroxyl (OH), aliphatic/aromatic hydrocarbons, carbonyl (C=O), aromatic ring structures, and carbon-nitrogen (C-N) bonds. The observed frequencies in the API + Excipients mixture are generally consistent with the individual API frequencies, indicating that the presence of excipients does not significantly alter the functional groups present in the API.

Physicochemical evaluation of Drug substance Dispersible Tablets:

Pre-compression evaluation:

Flow properties of the formulation:

Table 3: Pre-compression Evaluation

Formulation	LOD (%) at 105°C	Bulk Density (gm/ml)	Tapped density (gm/ml)	Compressibility index (%)	Hauser's ratio	Flow ability
Batch 1	0.357	0.357	0.456	21.71	1.27	Passable
Batch 2	0.394	0.394	0.468	18.78	1.18	Good
Batch 3	0.375	0.375	0.487	22.99	1.31	Passable
Batch 4	0.375	0.375	0.458	18.12	1.22	Fair
Batch 5	0.476	0.476	0.571	16.63	1.19	Fair
Batch 6	0.416	0.416	0.521	20.15	1.25	Fair

Table 4: Physicochemical evaluation of compressed tablets batch no. 1 to 6

Formulation	Average weight (mg)	Individual weight variation (mg)	Hardness (N)	Thickness (mm)	Disintegration Test (in Purified Water at temperature 15-25°C)	Friability
Batch 1	625.42	623.9-628.2	104-115	5.36-5.42	3 min 14 sec	0.18
Batch 2	625.12	624.6-627.2	102-115	5.30-5.35	2 min 57 sec	0.27
Batch 3	625.18	623.8-628.4	106-118	5.32-5.38	3 min 04 sec	0.24
Batch 4	625.92	624.5-626.1	100-115	5.27-5.35	1 min 33 sec	0.22
Batch 5	625.45	623.25-625.9	100-114	5.35-5.42	1 min 15 sec	0.20
Batch 6	625.10	624.3-626.4	102-110	5.36-5.41	1 min 45 sec	0.21

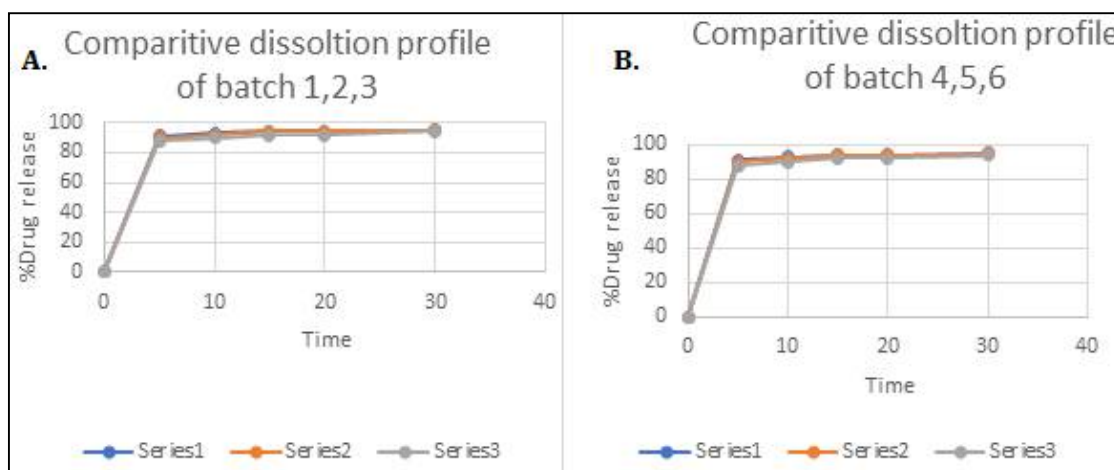


Figure 4: Comparative dissolution profile of Batch 1,2,3 and batch 4,5,6

Batch 4 exhibits the highest and most consistent drug release profile among the tested batches in the provided dissolution conditions. The presence of 0.4% SLS in the dissolution medium appears to enhance the drug's dissolution rate. However, further analysis and investigation are necessary to fully understand the dissolution behavior and optimize the formulation for desired drug release characteristics.

Content uniformity of tablets:

Table 5: Content uniformity of tablet from batch no. 1 to 6:

Sr No.	B1	B2	B3	B4	B5	B6
1	99.2	93.5	99.6	99.7	100.1	100.6
2	97.8	97.2	99.1	99.5	102.5	101.5
3	98.6	99.8	98.5	100.5	99.8	94
4	99.2	99.1	100.2	101.4	101.5	101.5
5	100.3	99.6	100.5	100.9	100.9	102.2
6	98.5	99.4	99.3	101.7	99.9	100.7
7	93.6	95.4	93.6	96.5	101.4	100.8
8	101	100.4	99.9	99.2	94.5	93
9	99.2	99.5	99.4	99.6	102.6	100
10	98.7	99.8	100.2	99.5	101.9	101.1
AVG	98.61	98.37	99.03	99.85	100.51	99.54
NO	10	10	10	10	10	10
K	2.4	2.4	2.4	2.4	2.4	2.4
SSD	1.982955	2.273055	1.998916	1.46534	2.341628	3.247632
RSD	2.010907	2.310719	2.018496	1.467542	2.329746	3.26264
AV	4.649092	5.585331	4.267399	2.166817	3.609907	6.754316

The content uniformity varies across the different batches (B1-B6). batches B5 and B6 exhibit the highest average content uniformity, while B2 shows the highest standard deviation and relative standard deviation. batch 4 shows the lowest standard deviation and relative standard deviation, indicating relatively consistent content uniformity. The acceptance values (AV) also vary across batches, with B4 having the lowest AV, indicating better content uniformity. Overall, further investigation and analysis may be required to determine the factors influencing content uniformity and to ensure compliance with desired standards.

DSC Study:

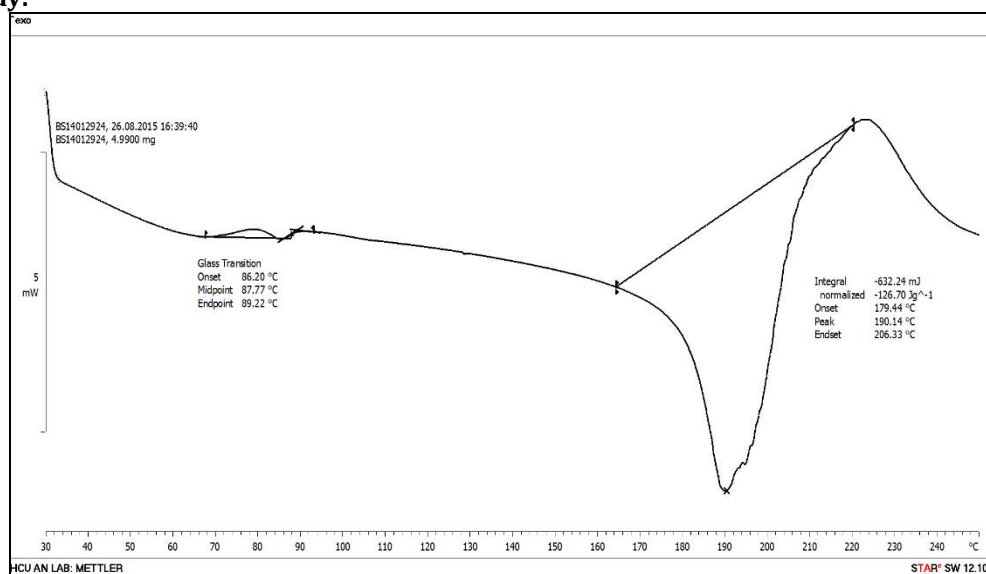


Figure 5: DSC plot for formulation

The DSC (Differential Scanning Calorimetry) plot of the API (Active Pharmaceutical Ingredient) exhibits three endothermic peaks at a maximum peak temperature of 190.42 degrees Celsius. These peaks indicate that the API undergoes three different processes or transitions at this temperature range. Moving on to the formulation, its DSC plot shows a glass transition temperature at 87.77 degrees Celsius, which suggests that the formulation undergoes a transition from a glassy or rigid state to a more amorphous or flexible state. This transition is typically associated with increased molecular mobility. Furthermore, as the temperature reaches 89.22 degrees Celsius, the formulation shows an end point where it exhibits an amorphous nature. This means that the formulation has fully transitioned into an amorphous or non-crystalline state, which can have implications for its stability, dissolution, or other properties. In conclusion, the DSC analysis of the API and formulation reveals important thermal characteristics. The API demonstrates three distinct transitions at a higher temperature, while the formulation exhibits a glass transition temperature and reaches an amorphous state. These findings provide valuable insights into the thermal behaviour and stability of the API and its formulation, which can be crucial for pharmaceutical development and manufacturing processes.

Stability study:

The final trial's stability experiments were conducted for the first, first, and third months by packing alu-alu blister packs in a humidity chamber (40°C/75% RH). All formulation parameters, including physical parameters, impurity profiles, content uniformity, and dissolution profiles, were within specification limits, according to the results shown in the table for the initial, first, and third months. Therefore, it suggests that the optimized formulation experiment is stable. The stability data suggests that the Alu-Alu blister pack provides adequate protection to the pharmaceutical product, maintaining its chemical stability, moisture barrier, structural integrity, and dissolution characteristics.

CONCLUSION

The conclusion drawn from the above data is that the formulation of dispersible tablets using the spray drying technique to achieve solid dispersion of the Active Pharmaceutical Ingredient (API) was successful in improving the dissolution and solubility characteristics of poorly soluble drugs. The presence of an amorphous nature of the API was confirmed through FTIR and DSC studies. However, the initial batches of the tablets showed slow disintegration, which was attributed to the insufficient application of the disintegrant, croscopolvidone. To address this issue and improve the tablet's disintegration time and overall effectiveness, several formulations were created, varying the concentrations of the binder, croscopolvidone (disintegrant), BHT (antioxidant), and magnesium stearate (lubricant). The optimal concentration of croscopolvidone was found to be 62.5 mg (10%), as it provided the tablets with appropriate properties for dissolution, disintegration, and hardness. The formulation with this concentration showed excellent dissolving, disintegration, and hardness within the desired range. Furthermore, the study on the short-term stability of the formulated dispersible tablets over three months showed no significant changes in the drug content, release profile, or outward appearance when stored at 40°C and 75% RH. Overall, the developed dispersible tablet formulation has promising release profiles, disintegration times, and

stability, making it a potentially effective and practical formulation for improving the bioavailability of poorly soluble drugs.

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