RESEARCH PAPER

Stabilization of Amorphous State of Genistein Using Porous Mango Seed Starch

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Abstract

The present study aimed to stabilize the amorphous form of the poorly water-soluble drug genistein (GNS) using porous mango seed starch (PMSS), a natural polymer obtained from *Mangifera indica* seed kernels. PMSS was prepared through a modified aqueous gelatinization–solvent exchange method, producing a porous matrix with a yield of **85.56 ± 2.45%**. Solid dispersions (SDs) of GNS with PMSS were developed using a solvent displacement–evaporation technique employing different acetone:water ratios (90:10–50:50 v/v). The formulations exhibited satisfactory recovery with yields ranging from **73.23 ± 1.25% to 80.56 ± 3.25%**. Among the tested systems, the **60:40 acetone:water ratio** produced the optimized dispersion with **drug loading of 95.36 ± 2.12%** and superior dissolution performance. Solid-state characterization using ATR-FTIR, ^1H-NMR, powder X-ray diffraction (PXRD), and differential scanning calorimetry (DSC) confirmed the transformation of crystalline GNS into an amorphous molecular dispersion within the PMSS matrix. PXRD showed disappearance of characteristic crystalline peaks of GNS, while DSC confirmed amorphization through the absence of the melting endotherm at **~299 °C**. Accelerated stability testing at **40 °C/75% RH for 90 days** showed no recrystallization, and dissolution after 6 h decreased only slightly from **98.65 ± 2.36% to 94.54 ± 1.45%**. The improved stability is attributed to molecular confinement within the porous PMSS matrix and intermolecular hydrogen bonding that restricts molecular mobility and inhibits recrystallization. Overall, PMSS demonstrated strong potential as an eco-friendly carrier for stabilizing amorphous drugs and enhancing dissolution of poorly water-soluble compounds.

Keywords: Porous mango seed starch; Amorphous stabilization; Solid dispersion; Drug–polymer interaction; Dissolution enhancement; Accelerated stability study

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Introduction

Currently, the development of amorphous solid dispersions (ASDs) has gained considerable attention as an effective strategy to enhance the solubility and dissolution rate of poorly water-soluble drugs due to their simplicity and economic feasibility (Serajuddin et al. 1988; Leuner and Dressman 2000). Being a high-energy state, the amorphous form is responsible for enhancement of dissolution and solubility as compared to the stable crystalline form. From a thermodynamic viewpoint, the amorphous form is a metastable state possessing higher free energy, which gives rise to physical instability and a tendency to recrystallize during storage over time (Ahlneck and Zografi 1990; Yoshioka et al. 1994; Zhou et al. 2002; Graeser et al. 2009) . Thus, the development of a stable amorphous form for successful drug delivery remains a major challenge for formulation scientists. Different strategies have been employed to stabilize the amorphous form of drugs such as storage below the glass transition temperature (Tg) (Hancock et al. 1995; Pokharkar et al. 2006), the anti-plasticizing effect of polymers (Matsumoto T, Zografi G 1999), and the promotion of intermolecular interactions between drug and polymer such as hydrogen bonding and/or electrostatic interactions (Pignatello et al. 2004a; Shakhtshneider et al. 2007).

Genistein (GNS) is a naturally occurring isoflavone widely reported for its antioxidant, anti-inflammatory, anticancer, cardioprotective and neuroprotective activities. However, GNS is a poorly water-soluble compound and exhibits low oral bioavailability due to its crystalline structure and limited aqueous solubility. Although conversion of crystalline GNS into its amorphous form can significantly enhance its apparent solubility and dissolution rate, the

amorphous form is thermodynamically unstable and prone to recrystallization, which may compromise its performance during storage. Therefore, stabilization of amorphous GNS is essential for improving its pharmaceutical applicability.

Literature survey reveals that stabilization of the amorphous state of drugs has been carried out using various polymers. Natural polymers such as moringa gum (Bhende and Jadhav 2012) and synthetic polymers including polyvinylpyrrolidone (PVP) (Van den Mooter et al. 2001), Eudragit® (Shan-Yang Lin et al. 1994; Pignatello et al. 2004a)), polyethylene glycol (Bley et al. 2010), hydroxypropyl cellulose (Sarode et al. 2014), and polymer combinations such as Eudragit®, Soluplus® and Kollidone® (Qi et al. 2013) and biopolymer such as sericin(Salunkhe et al. 2019) have been investigated for their potential as stabilizers of amorphous drug forms. In addition, mesoporous materials such as Upsalite (mesoporous magnesium carbonate) have also been explored for stabilization of amorphous systems (Zhang et al. 2014).

In the present investigation, porous mango seed starch (MSS), extracted from the kernels of *Mangifera indica*, has been explored as a novel natural stabilizing polymer. Mango seed kernel is an agro-industrial by-product rich in starch content and is often discarded as waste. The extracted starch exhibits excellent biocompatibility, biodegradability and hydrophilic characteristics. Due to the presence of abundant hydroxyl groups, starch can form strong intermolecular hydrogen bonding with drug molecules. Such interactions may reduce molecular mobility, inhibit nucleation, and prevent recrystallization of amorphous GNS (Zhang et al. 2014; Wang et al. 2019). Compared with plain (native) starch, PMSS offers superior stabilization of amorphous systems. Its well-developed pore architecture enables effective molecular confinement of GNS within internal cavities, significantly restricting molecular mobility and suppressing nucleation and recrystallization(Hancock et al. 1995; Ali et al. 2013; Zhang et al. 2014). Moreover, the abundant and accessible hydroxyl groups facilitate strong drug–polymer hydrogen bonding, contributing to thermodynamic stabilization (Matsumoto and Zografi 1999; Pignatello et al. 2004b; Farzan et al. 2023). Furthermore, the polymeric matrix may also elevate the Tg of the system and provide resistance against moisture-induced plasticization, thereby improving solid-state stability.

Looking at these advantages, the present study was undertaken to explore the potential of PMSS in stabilizing the amorphous state of GNS. Solid dispersions (SDs) of GNS with PMSS were prepared using solvent evaporation. The prepared SDs were subjected to various physicochemical characterizations including Fourier Transform Infrared Spectroscopy (FTIR) to study possible intermolecular interactions, X-ray Diffraction (XRD) to confirm amorphization, Differential Scanning Calorimetry (DSC) to evaluate thermal behavior and Tg, and in vitro dissolution studies to assess improvement in dissolution performance. This approach not only aims to enhance the solubility and dissolution rate of GNS but also promotes the utilization of an eco-friendly and economical natural polymer derived from mango seed waste, thereby contributing to sustainable pharmaceutical development.

Materials

Genistein (GNS) was obtained as a gift sample from a Dhamtek pharma (Mumbai, India). Mature mango seeds of Mangifera indica were collected from a local fruit processing source. Acetone and other chemicals and reagents were procured from Loba Chemi (Mumbai, India).

Methods

Preparation of porous mango seeds starch (PMSS)

PMSS was prepared using a modified aqueous gelatinization solvent exchange method reported previously (Ali et al. 2013; Wang et al. 2019). Briefly, 10 g of starch was dispersed in 40 mL of distilled water at ambient temperature to obtain a uniform suspension. Separately, 60 mL of distilled water was heated to boiling, and the starch dispersion was gradually introduced under continuous vigorous stirring. Stirring was maintained while the system was allowed to cool to room temperature, leading to the formation of a translucent starch gel. The obtained gel was immersed in excess distilled water and stored at 8 °C overnight to attain equilibrium. Subsequently, solvent exchange was carried out using acetone to preserve the porous network. The gel was equilibrated in acetone and maintained at 8 °C for 48 h. After solvent exchange, the material was dried in a hot air oven at 40 °C, pulverized to obtain a fine powder, and stored under vacuum until further use. The resulting product was designated as PMSS.

Loading of GNS by Solvent Displacement

Solid dispersions (SDs) of GNS with PMSS were prepared using a solvent displacement–evaporation method with slight modification of reported

procedures (Leuner and Dressman 2000; Paudel et al. 2013). Briefly, accurately weighed quantities of GNS were dissolved in acetone to prepare 25mg/mL, followed by the addition of distilled water to obtain binary solvent systems at varying volume ratios (90:10, 80:20, 70:30, 60:40, and 50:50 v/v). The drug solution was sonicated for 30 min to ensure complete dissolution and uniformity. The resulting solution was gradually incorporated into PMSS under continuous stirring and allowed to equilibrate for 6 h to facilitate drug adsorption and entrapment within the porous matrix. Subsequently, the solvent was removed by controlled evaporation at 40–50 °C until a dry mass was obtained. The dried product was gently scraped, pulverized using a mortar and pestle, and passed through a 60-mesh sieve to obtain uniform particle size. The prepared SDs were stored in a desiccator until further characterization.

Evaluation of Solid Dispersions

Drug Content

Accurately weighed SDs equivalent to 10 mg of drugs were dissolved in 100 ml of the acetone:water (60:40 v/v). The resulting solutions were kept for overnight and sonicated for 30 min for complete extraction of drug. The solutions were filtered, diluted suitably and drug content was analyzed by UV spectrophotometer. All measurements were performed in triplicate.

ATR-FTIR-infrared spectroscopy

The infrared spectra of GNS, PMSS, and their SDs were obtained using ATR-FTIR spectrophotometer (Bruker Alpha II ATR-FTIR spectrophotometer, Germany) in the region of 4000–400 cm^{-1}.

^{1}H-NMR

^{1}H-NMR experiments were carried out on prepared GN-MS SDs, and pure drugs to investigate the interaction between PMSS, and GNS using NMR spectrometer (Bruker) operated at 300 MHz, equipped with a 5 mm probe. Sufficient SD's/drugs sample was dissolved in solvent DMSO and then used for analysis.

Accelerated Stability Study

For optimization purposes, all prepared solid dispersions (SDs) were evaluated by in vitro dissolution studies, and the formulation exhibiting superior dissolution performance was selected as the optimized batch. Based on these results, the GN–MSS solid dispersion prepared using the acetone:water ratio of 60:40 (v/v) was selected for further stability evaluation. Accelerated stability studies of the optimized SD were conducted in accordance with ICH guidelines. The samples were transferred into tightly closed glass vials and stored at 40 °C ± 2 °C and 75% ± 5% relative humidity (RH). At predetermined intervals (0, 15, 30, 45, 60, 75, and 90 days), samples were withdrawn and analyzed using PXRD, DSC, and in vitro dissolution studies to assess the effect of elevated temperature and humidity on the physical stability of the GN–MSS solid dispersion.

Powder X-ray diffraction

Powder X-ray diffraction (XRD) pattern of the pure GNS, PMSS and GN-MS SDs were obtained by using Bruker, Germany. Model: D2 Phaser diffractometer with Cu Kα radiation (λ = 1.5405 Å) radiation and a crystal monochromator, with a voltage of 40 mV and a current of 30 A. The diffraction patterns run at 5-10° C min^{-1} terms of 2θ angles.

Differential scanning calorimetry (DSC)

DSC studies of the pure GNS, PMSS and GN-MS SDs were performed using a Hitachi STA7300 thermal analyzer equipped with Nexta Measure software (version 21112037A4-01). Indium/zinc standards were used to calibrate the DSC temperature and enthalpy scale. The samples were hermetically sealed into pierced aluminum pans and heated at a constant rate of 10°C/min over a temperature range of 35 to 500°C. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 mL/min.

In vitro drug release study

The dissolution studies of all SDs (equivalent to 50 mg of GNS) were performed under non sink conditions using a US Pharmacopeia type II dissolution test apparatus (Electrolab TDT-06P, Mumbai, India). The condition of dissolution test was as follows: medium – 900 ml phosphate buffer pH 7.4; speed – 100 rpm; temperature – 37 ± 0.5 °C. During the dissolution studies, samples of 5 ml were withdrawn at different time intervals from 30 min to 10 hrs and replaced with an equal volume of release medium. The samples were subsequently filtered and absorbance was measured at 260 nm. The experiments were performed in triplicate. Same procedure was used for optimised GN-MS SDs at

Result and Discussion

PMSS was successfully prepared using a modified aqueous gelatinization–solvent exchange method. During the process, starch granules underwent gelatinization when the starch dispersion was gradually added to boiling water with continuous stirring, leading to disruption of the crystalline regions and formation of a translucent gel network. Cooling and storage at 8 °C facilitated stabilization of the gel matrix through molecular rearrangement of PMSS chains. Subsequent solvent exchange with acetone for 48 h replaced the water present within the gel

network, which helped maintain the internal porous structure during the drying process. After drying and pulverization, PMSS was obtained with a percentage yield of 85.56 ± 2.45%. The slightly lower yield may be attributed to partial leaching of soluble amylose during gelatinization and washing, minor losses during solvent exchange and handling, adhesion of material to utensil walls during processing, and losses during transfer.

Loading of GNS by Solvent Displacement

GNS-loaded SDs with PMSS were successfully prepared using a solvent displacement–evaporation technique. The binary solvent system consisting of acetone and water played an important role in facilitating drug loading within the porous matrix. Acetone was used as a good solvent for genistein, while water promoted swelling of the porous starch structure, thereby enabling enhanced diffusion and entrapment of the drug molecules into the internal pores of PMSS. Different acetone–water ratios (90:10, 80:20, 70:30, 60:40, and 50:50 v/v) were investigated to optimize the solvent composition. The prepared dispersions showed percentage yields (Table 1) ranging from 73.56±3.25% to 80.23± 1.25%, respectively, indicating satisfactory recovery of the drug–carrier system using the solvent displacement–evaporation method. Minor variations in yield were likely due to handling losses during solvent evaporation, scraping, pulverization, and sieving processes.

Table 1 Percent yield of all batches of SDs

Sr. No.	Batch Code (Acetone:Water, v/v)	*Percent yield %
1	90:10	80.56±3.25
2	80:20	78.81±1.25
3	70:30	75.47±1.25
4	60:40	74.47±1.25
5	50:50	73.23± 1.25

*Values are expressed as the mean ± SD (n =3)

The optimization of the binary solvent composition was performed based on in vitro drug dissolution performance, as improved dissolution indicated more efficient drug incorporation and dispersion within the porous starch matrix. Among the investigated systems, the 60:40 v/v (acetone:water) solvent ratio exhibited the highest dissolution profile, suggesting more effective adsorption and distribution of GNS within the porous structure of PMSS. At this ratio, sufficient acetone was available to maintain GNS in dissolved form, while the presence of water promoted swelling of the porous starch network, facilitating deeper penetration and complex formation of the drug within the pores. In contrast, higher acetone content reduced swelling of the matrix, while higher water content decreased drug solubility, which could limit efficient loading. Therefore, the 60:40 v/v binary solvent system was considered optimal and was selected for further characterization studies.

Drug Content (%)

The percent drug content of all the batches are given in table 2. The SDs 60:40 v/v showed a drug content of 95.36±2.12%, indicating efficient incorporation of GNS into the PMSS carrier. The acetone:water (60:40 v/v) system effectively extracted the drug from the dispersion, further supporting successful drug loading. These findings indicate that 60:40 v/v (acetone:water) is a suitable for the incorporation of GNS into PMSS and may contribute to improved drug delivery performance.

Table 2 Percent of drug content

Sr. No.	Batch Code (Acetone:Water, v/v)	*Drug content %
1	90:10	80.56±3.25
2	80:20	78.81±1.25
3	70:30	75.47±1.25
4	60:40	74.47±1.25
5	50:50	73.23± 1.25

*Values are expressed as the mean ± SD (n =3)

ATR-FTIR-infrared spectroscopy

The ATR–FTIR spectrum of GNS shown in Figure 1. The spectrum exhibited characteristic absorption peaks confirming the presence of functional groups associated with its isoflavone structure. A broad peak observed at 3456.78 cm^{-1} and 3404.18 cm^{-1} was attributed to O-H stretching vibrations of phenolic hydroxyl groups. The peaks at 3089.97 cm^{-1} and 3030.61 cm^{-1} corresponded to aromatic C–H stretching vibrations, indicating the presence of aromatic rings. The bands at 2964.89 cm^{-1}, 2920.19 cm^{-1}, and 2830.71 cm^{-1} were associated with aliphatic C–H stretching vibrations. A prominent peak at 1664.97 cm^{-1} was assigned to the C=O stretching vibration of the conjugated carbonyl group present in the isoflavone backbone of GNS. The peaks appearing at 1578.69 cm^{-1}, 1504.49 cm^{-1}, and 1462.49 cm^{-1} were attributed to aromatic C=C stretching vibrations, confirming the phenyl ring structure of the compound. The absorption peaks at 1394.70 cm^{-1}, 1337.65 cm^{-1}, and 1294.75 cm^{-1} were related to O–H bending and C–O stretching vibrations of phenolic groups.

Overall, the observed ATR–FTIR peaks were consistent with the reported spectral characteristics of GNS, confirming the presence of hydroxyl, carbonyl, and aromatic functional groups and verifying the

structural integrity of the GNS. From the overlay of the ATR-FTIR spectrum of the GNS-PMSS solid dispersion, the characteristic peaks of GNS were retained but showed slight shifting and broadening, particularly in the O-H (~3400 cm^{-1}) and C=O (~1660 cm^{-1}) regions. These changes suggested the formation of intermolecular hydrogen bonding between the phenolic hydroxyl groups of GNS and the hydroxyl groups of the starch matrix. Minor variations observed in the C–O stretching region (1150–1000 cm^{-1}) further indicated interactions between the drug molecules and the polysaccharide framework of PMSS. In addition to hydrogen bonding, weak intermolecular interactions such as van der Waals forces between the aromatic rings of GNS and the polymeric backbone of PMSS may also contribute to drug stabilization. These non-covalent interactions are known to stabilize the amorphous form of poorly water-soluble drugs and prevent recrystallization in solid dispersion systems (Hancock and Zografi 1997; Leuner and Dressman 2000). The absence of new absorption bands indicated that no chemical modification occurred, confirming that genistein was physically dispersed and stabilized within the PMSS matrix.

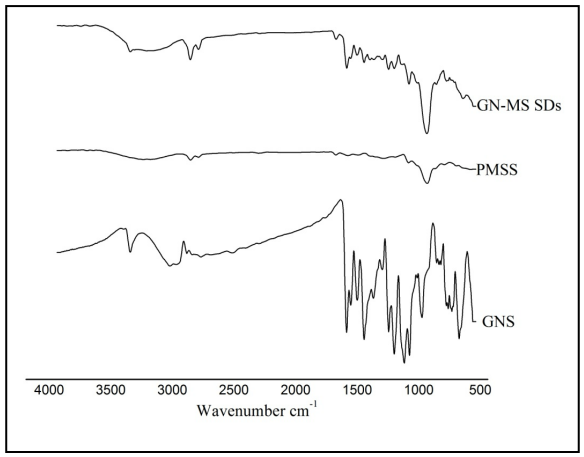

Figure 1 ATR-FTIR spectrum of GNS, PMSS and GN-MS SDs10.5.4. ^{1}H-NMR analysis

The ^{1}H NMR spectra of GNS, PMSS, and the GN–MS SDs are depicted in Figure 2. The ^{1}H NMR spectrum of GNS exhibited characteristic signals corresponding to the isoflavone framework. A strongly deshielded singlet at δ 12.96 ppm was assigned to the phenolic hydroxyl proton (5–OH), which arises due to intra-molecular hydrogen bonding with the adjacent carbonyl group. The A-ring aromatic protons appeared as meta-coupled signals at δ 6.22 ppm (H-6) and δ 6.38 ppm (H-8). The B-ring protons showed a typical AA′BB′ pattern, with doublets observed at δ 6.80–6.82 ppm (H-3′, H-5′) and δ 7.33–7.36 ppm (H-2′, H-6′), indicating the presence of a para-substituted phenyl ring. In addition, a singlet at δ 8.23–8.30 ppm corresponded to the H-2 proton of the heterocyclic C-ring, confirming the typical isoflavone structure of GNS (J.B. Harbome 1998; Yang et al. 2015; Chaitanya et al. 2022). The ^{1}H NMR spectrum of PMSS displayed broad signals mainly in the δ 3.20–5.20 ppm region, which correspond to protons of the glucose units (H-1 to H-6) in the starch polysaccharide backbone. The signal around δ ~5.1 ppm was attributed to the anomeric proton (H-1) of the glucose unit, while the resonances between δ 3.3–4.5 ppm corresponded to ring protons (H-2 to H-6) of the carbohydrate structure. The broad nature of these peaks is typical of starch due to overlapping proton environments and the polymeric nature of the polysaccharide(Ritota et al. 2008).

In the ^{1}H NMR spectrum of the GN–MS SDs, signals corresponding to both genistein and starch were observed, confirming the incorporation of the drug into the porous matrix. However, noticeable chemical shift variations and peak broadening were observed for several genistein signals. The phenolic hydroxyl proton originally appearing at δ 12.96 ppm showed reduced intensity and slight shifting, indicating participation in intermolecular hydrogen bonding with the hydroxyl groups of the starch matrix. Similarly, the aromatic proton signals at δ 6.22 ppm and 6.38 ppm (A-ring) and δ 6.80–6.82 ppm and 7.33–7.36 ppm (B-ring) exhibited slight broadening and reduced intensity in the complex spectrum. In addition, the H-2 proton signal at δ ~8.23–8.30 ppm showed minor chemical shift variation in the complex.

These spectral changes indicate that the local electronic environment of GNS protons was altered due to interactions with the starch matrix. The observed peak broadening and small shifts suggest the formation of intermolecular hydrogen bonding between the phenolic hydroxyl groups of GNS and the abundant hydroxyl groups present in PMSS. Furthermore, weak intermolecular interactions such as Van Der Waals attractive forces between the aromatic rings of GNS and the polymeric backbone of starch may also contribute to the stabilization of the drug molecules within the porous structure. The reduction in intensity and broadening of the aromatic signals, along with partial overlap with starch proton signals in the δ 3–5 ppm region, suggests restricted molecular mobility and molecular-level dispersion of GNS within the polymer matrix, which is a characteristic feature of amorphous solid dispersion systems. These interactions help prevent drug recrystallization and contribute to the stabilization of the amorphous form

of GNS within the porous starch carrier (Salunkhe et al. 2019).

Overall, the ^{1}H NMR results support the ATR–FTIR findings, confirming that the GNS interacted with PMSS by non-covalent intermolecular interactions such as hydrogen bonding and Van Der Waals forces, leading to the formation of a stable amorphous solid dispersion system.

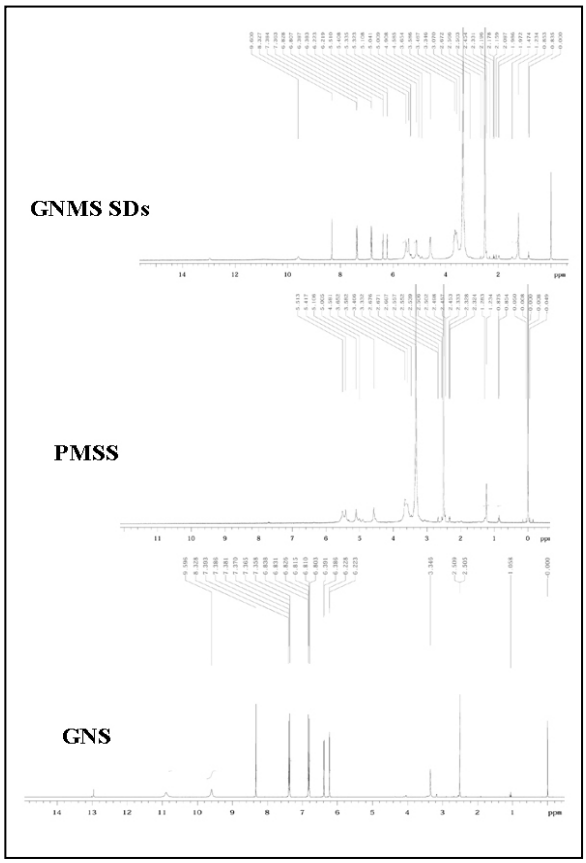

Figure 2 ^{1}H NMR spectrum of GNS, PMSS, and GN-MS SDs

Accelerated Stability Study

Powder X-ray Diffraction (PXRD) Analysis

The crystalline properties of GNS, PMSS, and the GN-MS SDs (60:30 v/v) were investigated PXRD (Figure 3). The PXRD pattern of pure GNS exhibited several sharp and intense diffraction peaks, confirming its highly crystalline nature. The major diffraction peaks were observed in the low 2θ region, particularly between 7° and 15°, indicating the presence of well-defined crystal lattice planes. A characteristic diffraction peak was observed at 2θ ≈ 7.62° with an intensity of 4563 counts, suggesting the presence of an ordered crystalline domain. A series of progressively increasing reflections were recorded between 2θ = 12.08° and 12.95°, with the most intense diffraction peak appearing at 2θ = 12.86° with a maximum intensity of 37,979 counts, representing the dominant crystallographic plane of GNS. Another moderate-intensity peak was detected at 2θ ≈ 14.45° (8677 counts). The presence of multiple sharp reflections and the absence of a diffuse halo confirm that GNS predominantly exists in a crystalline state. These diffraction peaks correspond to characteristic lattice planes reported for crystalline GNS in previously published PXRD studies and can serve as reference peaks for evaluating structural modifications or changes in crystallinity during formulation development(Wu et al. 2010; Hu et al. 2022)

The PXRD pattern of PMSS exhibited a broad and diffuse halo rather than distinct crystalline reflections, indicating the predominance of an amorphous structure. In the obtained diffractogram, a broad diffraction hump centered around 2θ ≈ 18–22° was observed, which is typically associated with the amorphous arrangement of starch polymer chains. The absence of intense and well-defined diffraction peaks suggests that the crystalline regions normally present in native starch granules were significantly disrupted during the pore-forming process. The relatively broad peak with moderate intensity indicates the presence of short-range molecular ordering but the absence of long-range crystallinity, confirming the transformation of starch into a partially or predominantly amorphous porous matrix. The amorphous nature of PMSS is advantageous for pharmaceutical applications because the increased surface area and disrupted crystalline domains enhance drug adsorption capacity and facilitate molecular dispersion within the carrier matrix(Zhu 2014; Vanier et al. 2016).

In contrast, the PXRD pattern of the GN-MS SDs (60:30 v/v) exhibited a significant reduction in the intensity of GNS characteristic crystalline peaks, along with their partial or complete disappearance. The diffractogram was dominated by a broad amorphous halo similar to that observed for PMSS, centered around 2θ ≈ 18–22°. The absence or substantial attenuation of the characteristic diffraction peaks of GNS in the 7–15° region indicates that the drug molecules were successfully incorporated into the PMSS matrix and were predominantly present in an amorphous or molecularly dispersed state. Compared with pure GNS, which displayed multiple sharp reflections indicative of a highly ordered crystalline lattice, the GN-MS SDs showed suppressed diffraction peaks and a broad halo pattern characteristic of amorphous materials.

The reduction in crystallinity can be attributed to the incorporation of GNS within the porous network of

starch, which restricts molecular packing and prevents the formation of an ordered crystal lattice. Such amorphization is commonly observed in solid dispersion systems, where the drug is stabilized in a disordered state within the carrier matrix. Consequently, the PXRD results confirm the successful transformation of GNS from a crystalline state to an amorphous or molecularly dispersed form within the PMSS carrier. This structural modification is often associated with improved physicochemical properties, particularly enhanced solubility and dissolution behavior of poorly water-soluble compounds such as GNS(Chiou and Riegelmant 1971; Vasconcelos et al. 2007).

The PXRD diffractograms of the optimized GN–MS SDs (60:40 v/v) obtained during the accelerated stability study did not show the appearance or growth of new crystalline peaks corresponding to GNS throughout the study period. The characteristic diffraction peaks of crystalline GNS remained absent or significantly suppressed in all samples, and the diffractograms were predominantly dominated by a broad diffuse halo associated with the amorphous structure of the PMSS matrix. The absence of peak growth indicates that no recrystallization of GNS occurred during storage under accelerated conditions. These observations suggest that the drug remained in an amorphous or molecularly dispersed state within the polymeric carrier throughout the stability testing period.

The inhibition of recrystallization can be attributed to the strong interactions between GNS and the PMSS. The porous structure of mango starch provides a large surface area that facilitates the molecular dispersion of the drug within the carrier network. Furthermore, the stabilization of the amorphous drug form is likely mediated by intermolecular interactions such as hydrogen bonding, van der Waals forces, and other intermolecular attractions between the hydroxyl groups of GNS and the functional groups present in the starch polymer chains. These interactions restrict molecular mobility and prevent the reorganization of GNS molecules into a crystalline lattice during storag(Vasconcelos et al. 2007; Liu et al. 2009).

Additionally, the confinement of drug molecules within the porous architecture of the starch matrix may further limit nucleation and crystal growth processes. As a result, the amorphous state of GNS remains thermodynamically stabilized within the polymer matrix even under elevated temperature and humidity conditions. DSC analysis also supported these findings, as no distinct melting endotherm corresponding to crystalline GNS was detected during the stability period, further confirming the absence of recrystallization.

Overall, the results of the accelerated stability study demonstrate that the optimized GN–MS SDs (60:40 v/v) solid dispersion exhibits good physical stability under accelerated conditions. The absence of crystalline peak growth in PXRD and the maintained amorphous characteristics indicate that the PMSS matrix effectively stabilizes GNS in a molecularly dispersed form, thereby preventing recrystallization during storage. This stability is expected to contribute to the sustained enhancement of solubility and dissolution behavior of GNS in the developed solid dispersion system.

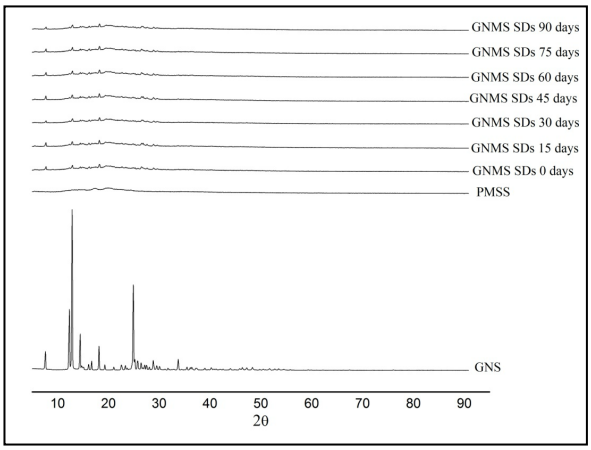

Figure 3 PXRD diffractograms of the GNS, PMSS and optimized GN–MS SDs

Differential Scanning calorimetry (DSC)

The thermal behavior and physical stability of the solid dispersion system were investigated using differential thermal analysis (Figure 4). The study involved evaluation of pure GNS, PMSS, and their optimised GN-MS SDs (60:30 v/v) in order to determine possible changes in crystallinity, drug–polymer interactions, and the stability of the amorphous state during storage. Thermal analysis is widely used to assess the physical state of drugs in solid dispersion systems and to detect drug–carrier interactions that may influence the stability of amorphous formulations.

The thermogram of pure GNS exhibited a sharp and intense endothermic peak at approximately 299 °C, corresponding to the melting point of crystalline GNS. The presence of this well-defined melting endotherm confirms the highly crystalline nature of the pure drug. The sharpness and intensity of the peak indicate a well-ordered crystal lattice and suggest the high purity of the compound. No additional thermal transitions were observed prior to the melting endotherm, indicating the absence of polymorphic

transformations before melting. The observed melting temperature is consistent with previously reported thermal characteristics of GNS, where a sharp endothermic peak between 298–302 °C has been associated with the melting of crystalline GNS (Tang and Pikal 2004).

In contrast, the thermogram of PMSS exhibited a markedly different thermal profile. A broad endothermic region was observed between 50–120 °C, which can be attributed to the removal of physically adsorbed and bound moisture present within the PMSS structure. PMSS contains numerous hydroxyl groups that readily interact with water molecules through hydrogen bonding, leading to the retention of moisture within the polymeric network (Ratnayake and Jackson 2009). Following this dehydration region, the thermogram did not display any distinct melting endotherm but rather showed a broad thermal transition between approximately 150–250 °C, which may correspond to structural rearrangements and partial degradation of the starch polymer chains. At higher temperatures, a gradual baseline shift was observed beyond 300 °C, which can be attributed to the thermal decomposition of the polysaccharide backbone, involving cleavage of glycosidic linkages and depolymerization processes (Liu et al. 2009). The absence of a sharp melting peak indicates the predominantly amorphous nature of PMSS.

A significant change was observed in the thermogram of the GN-MS SDs. The characteristic melting endotherm of GNS at approximately 299 °C completely disappeared in the thermogram of the SDs. Instead, the thermal curve exhibited a broad and smooth profile similar to that of the PMSS carrier, without any detectable melting transition corresponding to the crystalline drug. The disappearance of the melting peak strongly indicates that GNS no longer exists in its crystalline form within the formulation, but rather is present in an amorphous state.

The absence of the drug melting endotherm suggests that GNS is molecularly dispersed within the PMSS matrix, leading to the formation of an amorphous SDs. The amorphization of the drug can be attributed to several intermolecular interactions occurring between GNS and the PMSS. GNS contains multiple phenolic hydroxyl groups, while PMSS possesses abundant hydroxyl functionalities along its polysaccharide backbone. These functional groups facilitate the formation of intermolecular hydrogen bonds between the drug molecules and the polymer chains, which disrupt the ordered crystal lattice of GNS and stabilize the drug in a disordered amorphous form (Hancock and Zografi 1997; Vasconcelos et al. 2007). In addition to hydrogen bonding interactions, van der Waals forces and other weak intermolecular interactions between the aromatic rings of GNS and the polymer network may further contribute to the stabilization of the molecularly dispersed drug.

Furthermore, the porous architecture and high surface area of the PMSS matrix provide a confined microenvironment that restricts molecular mobility and prevents drug recrystallization. The internal pore structure facilitates uniform distribution of drug molecules throughout the polymeric matrix, thereby promoting the formation of a stable amorphous system. Similar observations have been reported in polymer-based solid dispersions, where the disappearance of the characteristic melting peak in DSC thermograms indicates successful conversion of a crystalline drug into an amorphous molecular dispersion (Vasconcelos et al. 2007).

The thermograms obtained during the accelerated stability study showed no significant changes in the thermal behavior of the solid dispersion throughout the storage period. Importantly, the characteristic melting endothermic peak of crystalline GNS remained absent in all thermograms even after 90 days of storage, indicating that the drug remained in a stable amorphous form within the PMSS matrix. Moreover, no new endothermic or exothermic peaks corresponding to recrystallization or polymorphic transformation were observed during the stability study.

The absence of recrystallization under elevated temperature and humidity conditions demonstrates that PMSS effectively stabilizes the amorphous form of GNS within the SDs system. Amorphous drugs generally possess higher free energy and greater molecular mobility compared to crystalline forms, which can result in thermodynamic instability and a tendency toward recrystallization during storage. However, PMSS can inhibit crystal nucleation and growth by restricting molecular mobility and stabilizing the amorphous phase through intermolecular interactions(Hancock and Zografi 1997).

The stabilization mechanism in the present system can be attributed to drug–polymer interactions, including intermolecular hydrogen bonding and van der Waals forces, between GNS and the hydroxyl groups of the PMSS. These interactions reduce the molecular mobility of the drug and prevent the reorganization of

molecules into a crystalline lattice (Salunkhe et al. 2019)

Additionally, the nanoconfinement effect provided by the PMSS further restricts molecular rearrangement and inhibits crystal growth.

The stability results obtained from DSC analysis are also supported by other solid-state characterization techniques, including ATR-FTIR spectroscopy, XRD, and ^{1}H NMR analyses. The absence of characteristic crystalline diffraction peaks in PXRD patterns and the spectral changes observed in ATR-FTIR and ^{1}H-NMR spectra confirm the presence of strong drug–polymer interactions and the molecular dispersion of GNS within the PMSS. Collectively, these findings demonstrate that PMSS effectively inhibits crystal nucleation and growth of GNS, thereby stabilizing the drug in its amorphous state.

Overall, the combined thermal and stability studies confirm the successful transformation of crystalline GNS into a stable amorphous molecular dispersion within the PMSS. The absence of recrystallization during the accelerated stability study indicates that PMSS functions not only as a carrier but also as an effective crystallization inhibitor, stabilizing the amorphous drug through hydrogen bonding interactions, intermolecular forces, and spatial confinement within its porous matrix. These findings highlight the potential of PMSS as a promising carrier for SDs formulations aimed at improving the solubility, dissolution rate, and physical stability of poorly water-soluble drugs.

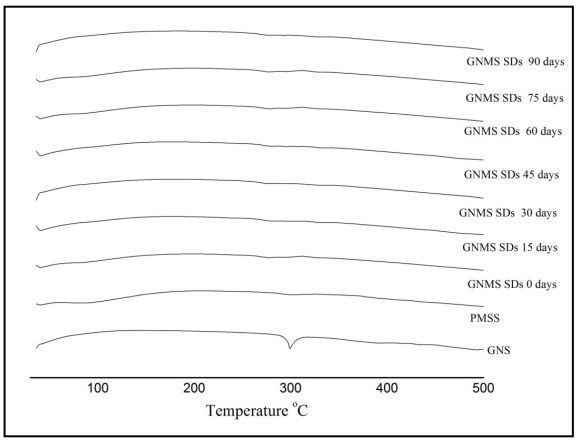

Figure 4 DSC Thermograms of pure GNS, PMSS and GNMS SDs

In-vitro drug dissolution study

The stability of the amorphous form of GNS in the PMSS system was evaluated through in vitro drug dissolution studies over a storage period of 90 days (Table 3). The percentage drug dissolution after 6 h remained nearly constant throughout the study period, with values ranging from 98.65 ± 2.36% at day 0 to 94.64 ± 1.45% at day 90. This minimal variation indicates that the dissolution behavior of GNS remained essentially unchanged during storage.

The dissolution profiles obtained at different time intervals (0–90 days) exhibited a similar pattern, suggesting that no significant physicochemical transformation occurred in the formulation. In amorphous drug systems, recrystallization during storage typically leads to a reduction in dissolution rate and extent of drug release, due to the formation of a more thermodynamically stable crystalline phase. However, the present results show no noticeable decline or alteration in the dissolution profile, indicating that GNS retained its amorphous state within the PMSS matrix throughout the study period.

The observed stability may be attributed to the porous architecture and high surface area of PMSS, which can effectively entrap drug molecules within its pore network. Such confinement restricts molecular mobility and prevents nucleation and crystal growth, thereby stabilizing the drug in an amorphous form. Additionally, possible hydrogen bonding or intermolecular interactions between GNS and PMSS chains may further contribute to maintaining the amorphous dispersion within the PMSS.

Therefore, the consistent dissolution performance over 90 days confirms that PMSS effectively stabilizes GNS in its amorphous state, preventing recrystallization during storage. This finding highlights the potential of PMSS as a promising natural carrier for enhancing the stability and dissolution performance of poorly water-soluble drugs.

Table 3 In Vitro Drug Dissolution Study of GN-MS SDs (60:30 v/v), After 6hrs

Time interval in days	In Vitro Drug Dissolution study (%)*	Conclusion
0	98.65±2.36	Amorphous
15	98.36 ±1.54	Amorphous
30	97.83 ±2.12	Amorphous
45	97.19 ±3.16	Amorphous
60	96.56 ±2.43	Amorphous
75	95.75 ±2.89	Amorphous
90	94.54 ±1.45	Amorphous

*Values are expressed as the mean ± SD (n =3)

Conclusion

The present study demonstrated the successful stabilization of amorphous genistein using porous mango seed starch as a natural carrier for solid dispersion formulation. PMSS prepared through a modified gelatinization–solvent exchange method

provided a porous polymeric matrix with a satisfactory yield of 85.56 ± 2.45%. Solid dispersions prepared by the solvent displacement–evaporation technique showed acceptable recovery (73.23–80.56%) and efficient drug incorporation. Among the evaluated formulations, the acetone:water (60:40 v/v) system produced the optimized dispersion with drug content of 95.36 ± 2.12% and the highest dissolution performance. Solid-state characterization confirmed the successful transformation of crystalline GNS into an amorphous molecular dispersion within the PMSS matrix. ATR-FTIR and ^1H-NMR analyses indicated intermolecular hydrogen bonding and weak non-covalent interactions between GNS and the hydroxyl groups of the starch polymer. PXRD patterns showed the disappearance of characteristic crystalline peaks of GNS, while DSC thermograms revealed the absence of the drug's melting endotherm at ~299 °C, confirming amorphization. Accelerated stability studies performed at 40 °C/75% RH for 90 days demonstrated excellent physical stability of the optimized formulation, with no detectable recrystallization in PXRD or DSC profiles. Dissolution performance remained largely unchanged, with drug release decreasing only slightly from 98.65% to 94.54% after 6 h, indicating preservation of the amorphous state during storage. The stabilization mechanism is likely associated with molecular confinement within the porous structure of PMSS and intermolecular interactions that reduce molecular mobility and inhibit nucleation. Overall, the results highlight the potential of porous mango seed starch as a sustainable, economical, and effective carrier for stabilizing amorphous drug systems and enhancing the dissolution behavior of poorly water-soluble compounds.

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