Journal of Applied Pharmaceutical Research
Volume 12 Issue 6, Year of Publication 2024, Page 192 – 202
DOI: https://doi.org/10.69857/joapr.v12i6.723

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Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

DEVELOPMENT OF HESPERIDIN SOLID DISPERSION FOR IMPROVED SOLUBILITY AND DISSOLUTION USING MANNITOL AND PVP K30 AS CARRIERS

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Article Information
Received: 22nd September 2024
Revised: 19th November 2024
Accepted: 15th December 2024
Published: 31st December 2024

Keywords
Hesperidin, Solid dispersion, Solubility enhancement, Dissolution rate, Kneading method, Solvent evaporation method

ABSTRACT
Background: Despite its six-hour half-life, Hesperidin, a bioflavonoid with therapeutic benefits, has low water solubility and bioavailability. This limits treatment. This study improved hesperidin solubility and dissolution by making solid dispersions using appropriate carriers. **Methodology:** Solid dispersions of hesperidin were prepared using two methods: kneading and solvent evaporation. The carriers utilized in the study were polyvinylpyrrolidone K30 (PVP K30) and mannitol. The formulations were evaluated based on various parameters, including yield, solubility, dissolution rate, drug content, and structural analysis using techniques such as X-ray diffraction (XRD), differential scanning calorimetry (DSC), and infrared (IR) spectroscopy. **Results:** Solid dispersions yielded 81.2% to 97.5% by weight and included 93.7% to 98.4% drug content. Hesperidin's solubility increased 3.72- to 24.05-fold, with a maximum drug release of 64.06% within 30 minutes. Comparatively, formulations with mannitol as the carrier demonstrated higher solubility (24.05 times) and dissolution (54.06%) than those containing PVP K30 (20.16 times and 34.36%). **Discussion:** Different carriers alter hesperidin solubility and dissolution. Mannitol improved drug release more than PVP K30. XRD and DSC experiments showed hesperidin's crystalline character changed in solid dispersions, possibly explaining its improved dissolving. IR spectroscopy showed physical dispersion because medication and carriers did not interact chemically. **Conclusion:** The study showed that solid dispersing hesperidin improves its solubility and dissolution. Drug release was greater with mannitol than with PVP K30. Solid dispersion formulations may improve the bioavailability of poorly soluble medicines like hesperidin.

INTRODUCTION

Hesperidin (Hesp) is 3',5,7-trihydroxy-4'methoxy flavanone 7-o-β-rutinoside, which is extensively found in citrus species and is the active constituent of tangerine peel [1]. It is associated with several pharmacological actions, including anti-inflammatory, antioxidant, anticancer activity, and hypotensive effects [2-5]. It prevents poisoning caused by lead strontium and heavy metals [6]. It is also used for diabetics and gastroesophageal reflux diseases and is helpful in the prevention of neurodegenerative diseases [7, 8]. The bioavailability of hesperidin is low due to its

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low aqueous solubility, absorption, and modification by microorganisms in the gastrointestinal tract and rapid excretion [9-11]. It is considered that the limiting step of the hydrolysis and absorption of hesperidin is the enzymatic activity α-rhamnosidase, which takes part in these processes. Although hesperidin is poorly absorbed and rapidly eliminated, it has a reasonable half-life of 6 h [12].

As previously mentioned, Hesp exhibits many pharmacological actions, but due to very low aqueous solubility, it shows poor dissolution and absorption. Considering the above factors that restrict the bioavailability of Hesp, there is an urgent need to solve these difficulties to enhance their availability and enable the use of their pharmacological potential in treating chronic diseases. Formulating hesperidin-chitosan complexes [13], inclusion complex of hesperidin with β-cyclodextrin [14-17], nanoparticles loaded with hesperidin [18-21], amorphous systems of Hesperidin with mesoporous material [22], nanocrystals [23-25], or eutectic mixtures [26] have been reported as approaches to enhance the bioavailability of hesperidin. Solid dispersion (SD) is a technique wherein the active pharmaceutical ingredient (API) is dispersed in an inert matrix or polymer.

It is widely accepted as an effective formulation technology to improve the dissolution and bioavailability of the molecules and mask the unpleasant taste of API by reducing particle size, creating high porosity and wettability, and forming amorphous API [27-32]. This investigation aimed to improve Hesp's solubility and dissolution rate by formulation as SD. Improvement in solubility and dissolution rate is expected to enhance the oral bioavailability of Hesp.

The literature reports the estimation of Hesp by UV-visible Spectrophotometric method [33] utilizing 0.1N NaOH solution as the solvent and a method using methanol as the solvent. Herein, we have used the method of Kuntic et al. (2012) using methanol and λ_{max} of 283 nm [34].

MATERIAL AND METHOD

Material

Hesperidine (>98%) was procured from Yarrow Pharmaceuticals, Mumbai, India. Polyvinylpyrrolidone (PVP) K30 (>99%, Himedia), mannitol (AR, Fisher Scientific), and methanol (LR, Sigma Aldrich) were used in the study.

Solubility Determination

The aqueous solubility of Hesp was determined by accurately weighing 10 mg of Hesp in a screw cap container and adding 20 mL of distilled water to it. The mixture was stirred at room temperature using a rotary flask shaker (Biotechnics, BTI-05A) for 24 h. The solution was left unperturbed for another 24 hours to equilibrate.

The solution was filtered through Whatman filter paper and suitably diluted with methanol to achieve concentration (5-25 µg/mL). Using a UV spectrophotometer (LT-2201, Labtronics, India) at 283 nm, this solution was analyzed using the calibration curve equation $y = 0.0059x + 0.0063$.

Preparation of Physical Mixtures (PM)

Accurately weighed quantity of Hesp (100 mg) and carriers (PVP and mannitol) in various drug-to-carrier weight ratios were thoroughly blended in glass mortar for 5 min.

Preparation of Solid Dispersion (SD)

The solid dispersion was prepared by varying the drug-to-carrier ratio (1:1, 1:2, and 1:4) and the carrier to ensure the best carrier and ratio for enhancing solubility and dissolution. The SD was prepared using solvent evaporation and kneading methods. While both these methods can effectively improve the solubility of drugs, the solvent evaporation method minimizes the risk of phase separation and thermal decomposition involved with methods like melt agglomeration. On the other hand, the kneading method possesses the advantages of uniform distribution of ingredients, lower drug-carrier interaction possibilities, and formation of an amorphous state of crystalline drugs.

In the solvent evaporation method, Hesp (100 mg) was dissolved in a minimum amount of ethanol in a beaker, and the required amount of carrier was added to it and mixed thoroughly at 40°C on a hot plate to obtain a clear solution.

The solvent evaporated, and the residue was scrapped off and sieved through sieve no. 100 [35]. The powdered SD was stored in air-tight containers for further studies (F1-F6). To prepare SD using the kneading method, Hesp (100 mg) and the required amount of carrier were accurately weighed and triturated using mortar and pestle to obtain the physical mixtures. A thick paste was prepared using a small volume of ethanol-water (1:1). The

paste was neatly kneaded and then dried at 45°C in a hot air oven (Biotechnics, BTI-29).

The dried mass was ground and sifted using a 24 mesh sieve, re-dried at 45°C, and finally sieved through 100 mesh to obtain a fine powder of SD (F7-F12) [36].

Determination of drug content in PM and SD

SDs/PM containing equivalent to 10 mg of Hesp was accurately weighed and transferred to a 100 mL Erlenmeyer flask, dissolved in a small volume of methanol, filtered, and transferred to a volumetric flask (100 mL), and made up to the volume with methanol. The solution was further diluted to obtain a 10 µg/mL concentration. The absorbance of this solution was measured at 283 nm against methanol as blank, and the drug content was calculated using the calibration curve equation y = 0.0053x + 0.0069.

Saturation Solubility Study

Saturation solubility measurements were conducted to evaluate the increase in Hesp's solubility in the SDs and the PM. A known excess (approximately 10 mg) of Hesp was added to 100 mL of phosphate buffer (pH 6.0). Samples were rotated at 200 rpm and 25 °C for 24 h, then filtered, suitably diluted, and analyzed by UV spectrophotometer at 283 nm (Adeli and Mortazavi, 2013).

X-ray diffraction studies of SDs and PM

The X-ray diffraction (XRD) of Hesp, PM, and SD was studied on X'pert Pro (PANalytical, Netherlands) using Ni-filter and CuKα1 radiation with Spinner PW3064. A 45 kV voltage and 40 mA current were applied with a scintillation counter. The XRD instrument scanned the samples from 5° to 80°.

Thermal Analysis

The thermal analysis of Hesp, PM, and SD was carried out using Differential Scanning Calorimetry (DSC) performed on a DSC instrument (Jade DSC, Perkin) equipped with Pyris 6 DSC software. The sample was pressed in an aluminum pan disc and placed on the instrument's heating head. The temperature of the DSC furnace was gradually increased from 30°C to 300°C, and the thermogram was obtained.

Fourier transform infrared spectroscopy (FTIR)

The FTIR studies of Hesp, PM, and SD were performed using an FTIR Spectrophotometer (Shimadzu, Japan). All samples were scanned from 400 to 4000 cm^{-1}.

Dissolution study

The dissolution rate for Hesp, PM, and SDs (F1-F12) was studied using a USP type II dissolution rate test apparatus in 900 mL of phosphate buffer (pH 6.0) at 37.0 ± 0.5 °C, and 100 rpm [37, 38] 100 mg of pure drug and an equivalent amount of preparations were accurately weighed and placed in dissolution flask of the apparatus.

Aliquots of 5 mL were withdrawn at specified intervals of 5, 10, 15, 20, and 30 min and replaced with fresh media. The samples were filtered with Whatman filter paper and analyzed spectrophotometrically at 283 nm for the dissolved drug. The dissolution studies were performed in triplicate.

Statistical Analysis

The study was carried out in triplicate, and the data expressed as (mean ± standard deviation) was subjected to an independent sample T-test using Graph Pad Prism software v 5.01 with a significance of $p<0.05$.

Results and Discussion

The calibration curve equation, y = 0.0059x+0.0063 in methanol, where x is concentration and y is absorbance, was used for the determination of the concentration of hesperidin in samples. The aqueous solubility of hesperidin was found to be 0.003 mg/mL, which increased by 66.66 times and 61.11% on preparing a physical mixture with PVP K30 and mannitol, respectively. The improved wetting of hesperidin due to intermolecular hydrogen bond formation between the carrier and hesperidin might be one of the reasons for improved solubility in the physical mixture [39].

Preparation of solid dispersion

The solid dispersion was prepared using kneading and solvent evaporation methods. These methods' foremost advantage is the avoidance of drug decomposition due to the requirement of low temperatures [40].

On the other hand, the kneading method minimizes the residual solvent in preparation owing to the minimal use of the organic solvent [41]. A total of six formulations using each method were prepared using PVP and mannitol as the carriers (Table 1). All the 12 formulations (F1-F12) were characterized for yield, drug content, solubility, and dissolution. The solid dispersion was obtained in a yield of 81.2 to 97.5% by weight. The high yield

suggests that a proper binary mixture of the drug and the carriers was used.

The drug content was determined in the solid dispersion to confirm the amalgamation of the drug and the carrier. The drug content in the formulations ranged from 93.7 to 98.4%. The yield and drug content were slightly higher in the kneading method compared to the solvent evaporation method (Figure 1).

Table 1: Ratio of the carrier in formulations

Batch Code	Ratio (drug to carrier)	
	PVP K30	Mannitol
Solvent evaporation		
F1	1:01	-
F2	1:02	-
F3	1:04	-
F4	-	1:01
F5	-	1:02
F6	-	1:04
Kneading		
F7	1:01	-
F8	1:02	-
F9	1:04	-
F10	-	1:01
F11	-	1:02
F12	-	1:04

Saturation Solubility Study

The increase in hesperidin's solubility by formulating it as a solid dispersion was studied using the method of Adeli and Mortazavi [35]. The PM enhanced the solubility of hesperidin by 1.61 times (Mannitol) and 1.66 times (PVP). All the SD formulations increased the solubility of hesperidin by 3.72 to 24.05 times (Table 2).

The highest solubility was exhibited by F6 (24.05 ± 6.44 times), whereas the lowest solubility was exhibited by F1 (3.72 ± 1.51 times). The higher solubility in SD can be attributed to forming a soluble complex between the drug and the carrier. In contrast, in the case of PM, the solubility of the drug is lower than SD, suggesting that the drug is still in its crystalline state in the PM [42].

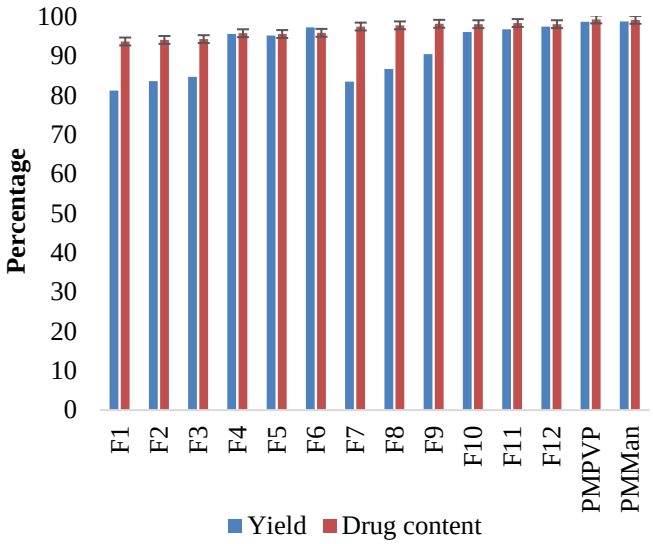


Figure 1: Yield and drug content of solid dispersion

It could be inferred from the result of solubility enhancement that increasing the carrier ratio led to an increase in the solubility of hesperidin. Such a carrier concentration-based increase in solubility was also witnessed in our previous study on azithromycin (Swarup and Agrawal, 2024) and also a study on SD of ketoprofen [44].

It could also be inferred that the method used to formulate SD impacted solubility enhancement. SD prepared using the solvent evaporation method exhibited higher solubility than those prepared using the kneading method. A similar effect of carrier ratio on the solubility of hesperidin has been reported in a previous study where Soluplus®, sodium alginate, and hydroxy propyl methyl cellulose, when used in 1:5 ratios, improved solubility by around 301 folds [45].

XRD and Thermal analysis of SD

X-ray powder diffraction (XRPD) is useful for identifying the difference between crystalline and amorphous phases. The diffractogram of Hesperidine exhibited sharp peaks at 12.11°, 15.43°, 19.48°, 21.33°, 22.54°, and 24.53°, 33.52°, 35.18°, 37.36° and 43.49° 2θ (Figure 2a).

The well-defined crystalline peaks of Hesperidine were less prominent in the PM after 24° 2θ, suggesting partial amorphization of the drug. In the SD, the occurrence of much smaller crystal peaks suggesting nanosization and the post 22° the diffractogram exhibited typical pattern of amorphous material with no distinct peaks.

Table 2. Solubility enhancement of hesperidin in PM and SD

Formulation	Solubility (mg/mL)	Increase in solubility (%)	Increased solubility (Times)
Pure Hesperidine	0.003 ± 0.0005	-	-
PM with PVP	0.005 ± 0.0005	66.66 ± 33.33	1.66 ± 0.3333
PM with Mannitol	0.004 ± 0.0011	61.11 ± 9.62	1.611 ± 0.0962
F1	0.011 ± 0.001	333.33 ± 145.29	4.33 ± 1.4529
F2	0.034 ± 0.0052	1233.33 ± 437.16	13.33 ± 4.3716
F3	0.052 ± 0.001	1916.66 ± 462.18	20.16 ± 4.6218
F4	0.013 ± 0.0005	422.22 ± 153.96	5.22 ± 1.5396
F5	0.043 ± 0.0011	1577.77 ± 365.65	16.77 ± 3.6565
F6	0.062 ± 0.0011	2305.55 ± 644.70	24.05 ± 6.4471
F7	0.009 ± 0.0015	272.22 ± 154.85	3.72 ± 1.5485
F8	0.035 ± 0.0005	1244.44 ± 307.92	13.44 ± 3.0792
F9	0.046 ± 0.001	1683.33 ± 404.48	17.83 ± 4.0448
F10	0.012 ± 0.001	372.22 ± 154.85	4.72 ± 1.5485
F11	0.04 ± 0.001	1461.11 ± 423.71	15.61 ± 4.2371
F12	0.058 ± 0.001	2161.11 ± 596.82	22.61 ±5.9682

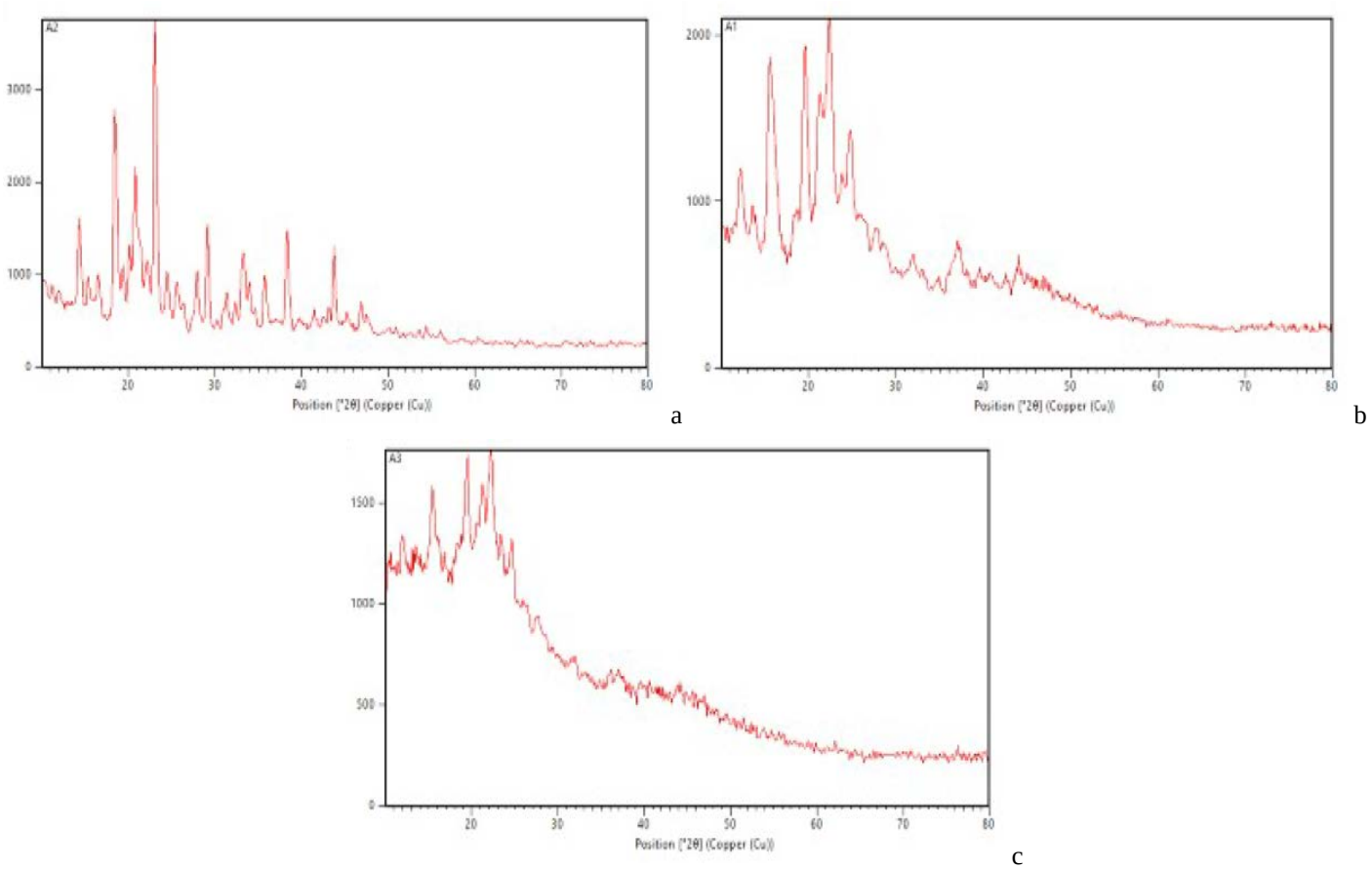
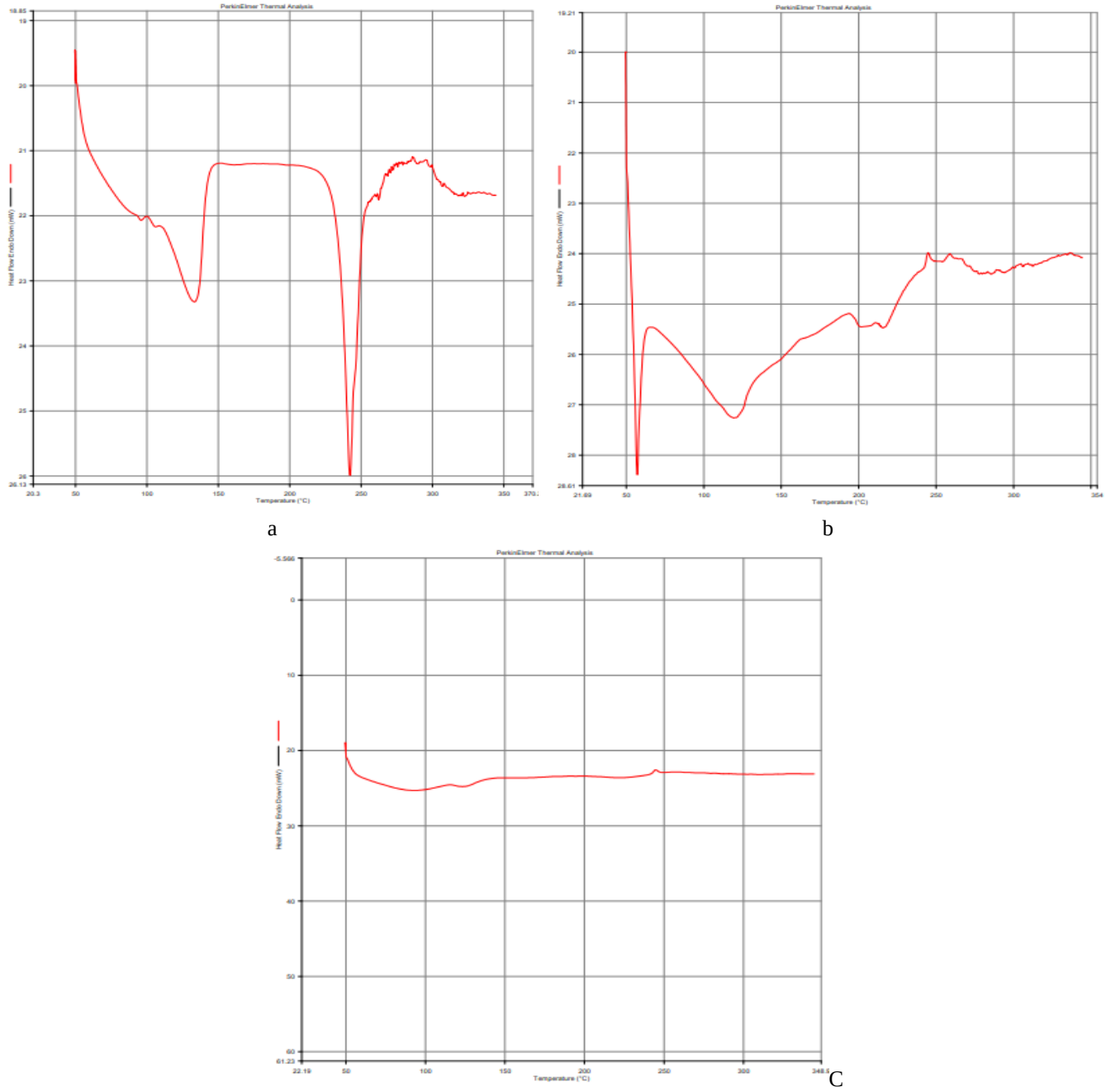


Figure 2. X-ray diffractogram of (a) Hesperidine (b) PM (c) SD

The DSC analysis of hesperidin revealed a glass transition temperature (Tg) of around 250°C (Figure 3a). In the thermogram of the PM, the Tg shifted to 120° (Figure 3b), whereas in the SD, no endotherm was obtained, explaining the formation of the amorphous binary mixture (Figure 3c). Studies have correlated the reduction in glass transition temperature to amorphization. The rapid initial decrease in Tg occurs due to the absorption of water followed by gradual leveling off at higher water contents, suggesting improved solubility on complete amorphization [46].



a b C

Figure 3: DSC Thermogram of (a) Hesperidine, (b) PM, (c) SD

FTIR study

Fourier-transform infrared spectroscopy analysis was performed to identify the major vibrations of hesperidin and the possible interactions between the carriers and hesperidin. The FTIR spectra of hesperidin displayed bands in the region of 800-1100 cm^{-1}, 1200-1500 cm^{-1} and 2300-2900 cm^{-1} corresponding to stretching of a C-O, aromatic C=C and C-H (Figure 4a). The spectra of the PM and SD also displayed the vibrations corresponding to the functional groups of hesperidin (Figure 4b and 4c). This suggests no interaction among the carriers and hesperidin.

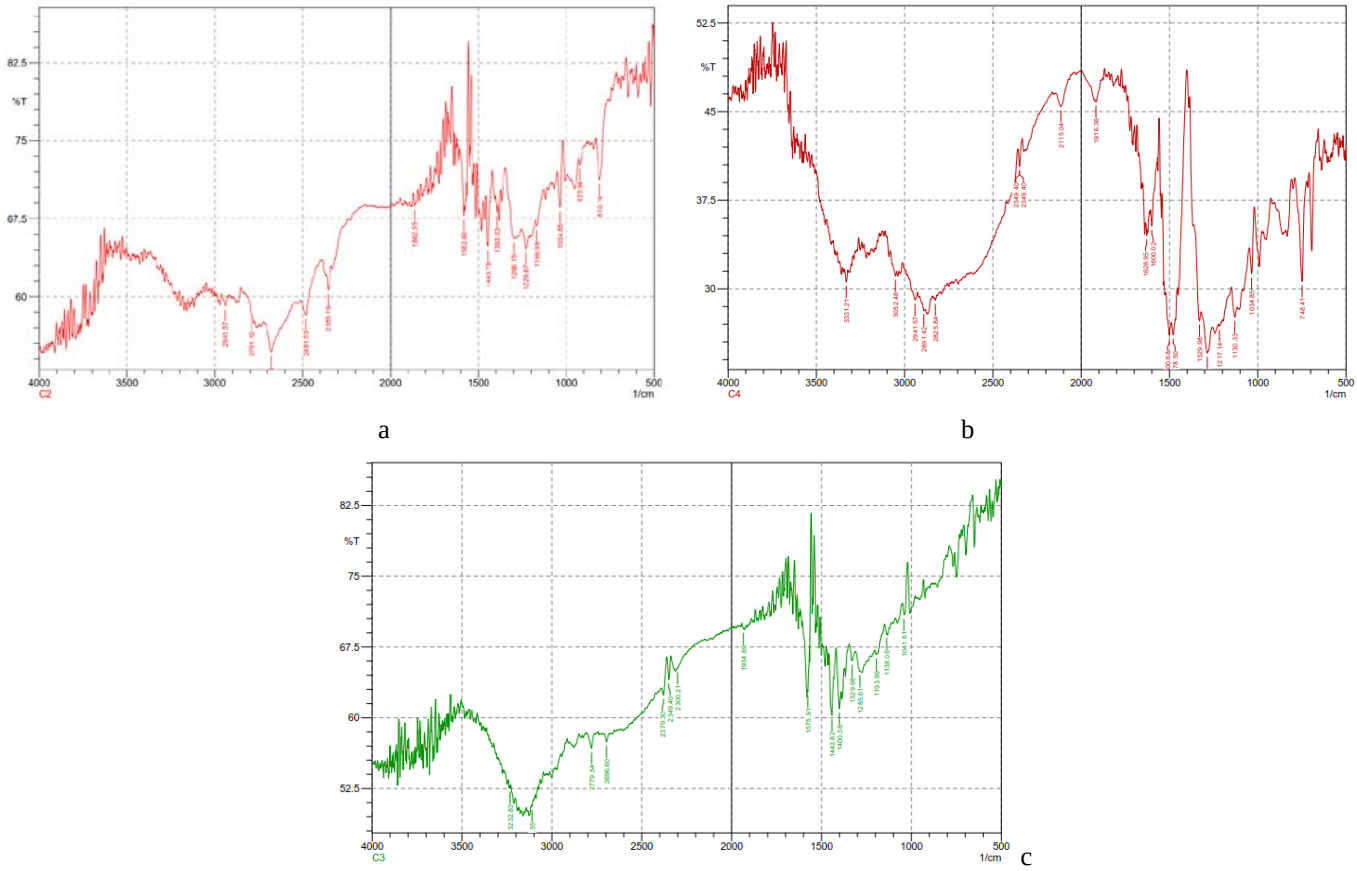


Figure 4: FTIR spectra of (a) hesperidin, (b) PM and (c) SD

Dissolution study

The *in vitro* dissolution study of the SD was carried out for 30 min, and the amount of hesperidin released was determined (Table 3). It was observed that 14.40 -64.06% hesperidin was released from the SD at the end of 30 min of the study. On the other hand, only 2.56% pure hesperidin was found in the same solution for the same duration. The highest amount of hesperidin was found to be released from F6 (64.06 ± 1.422%), whereas F1 released the least amount of hesperidin (14.40 ± 0.458%). The study also revealed that increasing the carrier ratio increased the release of hesperidin.

In a similar study conducted previously, 82.15 ± 0.07 % hesperidin was found to release in 0.1N NaOH solution in 30 min from SD prepared using Ocimum mucilage and mannitol mixture (7:3) as the carrier for SD [47]. The release of hesperidin in the present study was also affected by choice of carrier. It was found that mannitol, when used as a carrier, led to higher release than PVP (Figure 5). The improvement in the dissolution rate of hesperidin from the SD could be attributed to a decrease in the crystallinity of the drug and its molecular and colloidal dispersion in the hydrophilic carrier matrix [48].

DISCUSSION

The discussion section of the research article highlights several key findings regarding the solubility and dissolution rate of hesperidin when formulated as solid dispersions. It emphasizes that the carrier choice significantly influences the drug's solubility and release profile. Specifically, mannitol was more effective than PVP K30 in enhancing hesperidin's solubility and dissolution rates. The better solubility displayed by mannitol-based SD can be attributed to improved wettability of drug particles that occurs by attachment of mannitol to the surface as well as the ability of mannitol to form strong hydrogen bonds with water [49-50]. Also, in the solvent evaporation method, the reduction in particle size that occurs while preparing the solid solution helps prevent particle agglomeration, contributing to improved dissolution [51].

The researchers noted that the solid dispersion formulations led to a marked increase in the solubility of hesperidin, with enhancements ranging from 3.72 to 24.05 times, depending on the formulation. This improvement is attributed to the reduction in the crystallinity of the drug and its molecular and colloidal dispersion within the hydrophilic carrier matrix. The probable

mechanism of improved solubility and release from amorphous SD could be carrier-controlled, wherein continuous diffusion of the API from the carrier matrix into the release medium occurs, maintaining the desired concentration in SD and the solution to prevent drug crystallization [52]. Structural analysis techniques such as X-ray diffraction (XRD) and differential scanning calorimetry (DSC) revealed changes in the crystalline nature of hesperidin, which likely contributed to the improved dissolution behavior.

Additionally, infrared (IR) spectroscopy confirmed no significant chemical interactions between the drug and the carriers, indicating that the enhancement in solubility was due to physical dispersion rather than chemical modification. The findings suggest that solid dispersion formulations could be a viable strategy for improving the bioavailability of poorly soluble drugs like hesperidin, ultimately enhancing their therapeutic efficacy. Studies on improving solubility using PVP and PEGs have reported similar results with a concentration-dependent increase in solubility of the risperidone [53]. The study by Joshi et al. [47] also aligned with our results, where a blend of Ocimum mucilage and mannitol displayed a carrier concentration-dependent increase in the solubility of hesperidin.

Table 3. Release of hesperidin from SD in phosphate buffer pH 6.0

Time (min)	Cumulative Release (%)					
	0	5	10	15	20	30
F1	0	1.26 ± 0.208	3.93 ± 0.416	6.66 ± 0.379	10.56 ± 0.651	14.40 ± 0.458
F2	0	3.40 ± 0.658	9.63 ± 0.551	15.10 ± 0.557	21.43 ± 1.168	29.36 ± 0.902
F3	0	5.73 ± 0.709	16.03 ± 1.882	22.23 ± 1.206	27.56 ± 0.929	34.36 ± 1.026
F4	0	4.50 ± 0.529	11.36 ± 0.907	16.93 ± 0.737	28.13 ± 1.012	43.43 ± 1.665
F5	0	5.76 ± 0.764	15.10 ± 0.2	27.36 ± 0.839	39.60 ± 0.954	53.93 ± 4.045
F6	0	7.63 ± 0.462	22.76 ± 1.514	34.46 ± 0.513	51.00 ± 2.095	64.06 ± 1.422
F7	0	1.66 ± 0.208	4.43 ± 0.416	7.03 ± 0.902	11.13 ± 0.651	16.06 ± 0.252
F8	0	4.13 ± 0.404	10.46 ± 0.603	16.3 ± 0.529	23.06 ± 0.493	31.26 ± 0.586
F9	0	6.96 ± 0.321	17.26 ± 1.172	24.66 ± 0.981	31.13 ± 0.907	36.70 ± 1.4
F10	0	2.83 ± 0.252	6.50 ± 0.608	11.73 ± 0.734	16.20 ± 1.054	22.83 ± 1.159
F11	0	6.43 ± 0.289	16.33 ± 1.124	28.83 ± 0.751	37.20 ± 0.755	44.86 ± 1.124
F12	0	9.13 ± 0.850	21.43 ± 0.503	33.86 ± 0.751	45.96 ± 1.201	59.20 ± 0.656
pure Hesp	0	0.13 ±0.058	0.6 ± 0.100	1.33 ± 0.208	1.76 ± 0.153	2.56 ± 0.251
PMPVP	0	0.41 ±0.003	1.40 ± 0.033	2.53 ± 0.001	4.91 ± 0.006	6.16 ± 0.110
PMMan	0	0.32 ± 0.011	1.14 ± 0.110	1.72 ± 0.003	3.35 ± 0.004	5.43 ± 0.033

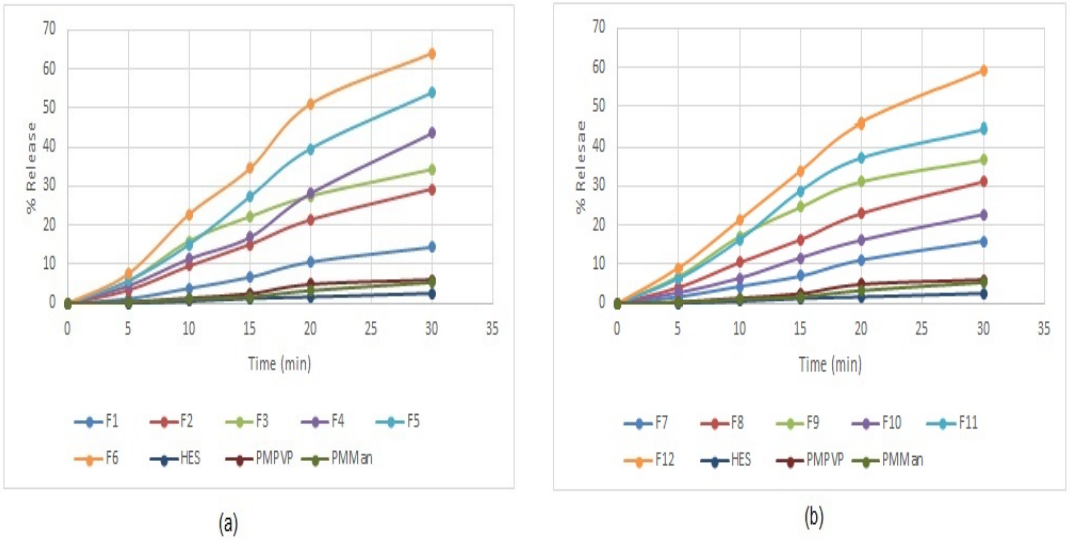


Figure 5: Release of hesperidin from SD

CONCLUSION

The low aqueous solubility of hesperidin hinders its therapeutic potential despite its significant health benefits. However, this study concludes that the solid dispersion approach offers an effective solution to improve hesperidin's solubility. Formulating solid dispersions using mannitol and PVP K30 carriers significantly enhances solubility and dissolution rate. Moreover, the solvent evaporation technique proves superior to the kneading method, yielding higher drug content and better release of hesperidin from the solid dispersions. Increasing the carrier ratio further improves solubility, suggesting that formulation methods play a critical role in overcoming the challenges of hesperidin's low bioavailability. This improvement is crucial for maximizing hesperidin's pharmacological potential in treating various chronic diseases. The formulation of Hesp as SD could help prepare a homogenous drug solution with higher stability, facilitating better clinical use.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Pallavi Swarup performed the experiments, collected data for analysis, and wrote the first draft of the manuscript. Gopal Prasad Agarwal conceptualized and revised the draft manuscript. Both authors read and approved the final manuscript.

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