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Formulation and Evaluation of Solid Dispersions of Poorly Water-Soluble Drug- Hesperidin

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Abstract: Hesperidin is a plant-derived flavonoid, abundantly present in the different citrus species, including lemon, orange, lime, and grapefruit. It possesses the diverse biological potential of therapeutic significance, including anti-inflammatory, anti-adipogenic, antioxidant, insulin-sensitizing, antimicrobial, neuroprotective, and anti-carcinogenic activities. The objective of the present study was to enhance the dissolution characteristics of the model drug by increasing the solubility and release rate of hesperidin through solid dispersions using natural polymers by the hot-melt extrusion method. The compatibility analysis was carried out through Fourier Transform-Infrared Spectroscopy (FT-IR) and Differential scanning calorimetry (DSC). The kinetic studies for drug release mechanisms were characterized through zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell models. The dissolution analysis of solid dispersions showed exhibited more than 99% drug released. The optimized formulation was found to follow the Higuchi model of drug release kinetics with an R^2 value of 0.919. Solid dispersions containing natural polymers prepared through the hot-melt extrusion method exhibited significant enhancement in the release profile compared to a pure drug, hesperidin.

Keywords: hesperidin; solid dispersions; natural polymers; ocimum mucilage; hot-melt extrusion; solubility enhancement.

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1. Introduction

Hesperidin is extracted on a large scale from *Citrus sinensis* L. and *Citrus unshiu* Marcovitch [1-3]. It has also been recognized in a variety of plant species, including Cyclopia (Fabaceae), Carpinus (Betulaceae), Mentha (Lamiaceae), and Pterocarpus (Papilionaceae) species [4-7]. In addition, hesperidin has also been reported in the bark of *Zanthoxylum avicennae*, *Zanthoxylum cuspidatum*, and roots of *Acanthopanax setchuenensis* Harmsex Diels [8, 9]. It was found to possesses diverse biological actions of therapeutic significance, like insulin-sensitizing, [10] anti-inflammatory, anti-carcinogenic activities [11], neuroprotective [12], type-2 diabetes [13], and metabolic syndrome [14], antioxidant and locomotor enhancing activities [15].

Chemically, it is (S)-7-[[6-O-(6-deoxy-alpha-L-mannopyranosyl)-beta-D-glucopyran osyl]-oxy]-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-Benzopyran-4-one, a colorless to a yellow crystalline powder having 260ºC melting point. Pure hesperidin occurs

as long hair-like needles, tan or pale yellow, tasteless and odorless powder [4]. The molecular formula of hesperidin is C28H34O15, with a molecular weight of 610.57 daltons. It is soluble in pyridine and dilutes alkali, forming a clear yellow solution, slightly soluble in hot glacial acetic acid, methanol, and almost insoluble in benzene, acetone, and chloroform. It has low aqueous solubility, i.e., 1 in 50; however, it is much more soluble in water than its aglycone hesperetin [16, 17].

Bioavailability is a major stage for guaranteeing hesperidin bioefficacy. The absorption rate of this drug can be enhanced by the implementation of various physical, chemical, and miscellaneous modification methods which are indulged in improving the solubility of BCS class II drug including complexation with chitooligosaccharide (COS) [18], the addition of co-solvents [19], supersaturation [20], the addition of adjuvants such as surfactants [21], drug dispersion in carriers like transglycosylated materials [22] etc. However, it gets rapidly metabolized into an inactive form, which lessens the calculated data of Cmax, Tmax, and AUC. The microbiota of the intestine (colon) converts hesperidin to hesperetin, where colonocytes absorb it via transcellular passive transport and proton-coupled active transport (colon) [23]. After absorption in the intestinal epithelium and liver, hesperidin is primarily excreted into bile and urine as glucuronide and/or sulfated conjugates [24]. The oral high-dose administration of hesperidin is considered cautious in various phases of clinical trials. The recommended dose was found to be 500mg, 2 times a day for 6 weeks to one year, causing minor adverse effects [25].

Solid dispersion is a method that increases the bioavailability of a drug by incorporating it into a polymer matrix, and nanoparticles are formulated to enhance the surface area of a drug [26]. The procedure was recorded for the preparation of the hesperidin drug. Solid dispersion is executed using natural polymer, i.e., Ocimum mucilage, through the melt extrusion method. The characterization of solid dispersion was performed using FTIR, DSC, and SEM [27]. The stable amorphous solid dissolution form of hesperidin is proved as the finest configuration with an enhanced dissolution rate.

2. Materials and Methods

2.1. Drug and chemicals.

Hesperidin was obtained as a gift sample from Hindustan Farm Direct Pvt. Ltd., Una, Himachal Pradesh. The polymer Ocimum mucilage was prepared in the laboratory. All other chemicals like Mannitol, NaOH, Ethanol, HCl were procured from Nice chemical Pvt. Ltd. and were of analytical grade.

2.2. Pre-formulation studies.

2.2.1. Detection of melting point.

The melting point of the drug was detected by the digital melting point apparatus through the open capillary method [28].

2.2.2. UV spectroscopy of the drug and calibration curve preparation.

The determination of absorbance maxima (λmax) was carried out by scanning the hesperidin solution (in 0.1N NaOH, 0.1 N HCl, ethanol, and water) between 200-400 nm, and the calibration curve was prepared on UV-1800 Shimadzu UV spectrophotometer [29]. In

statistics, the coefficient of determination, R^2 is used in statistical models to predict future outcomes based on other related information.

2.2.3. Solubility analysis of the drug.

An excess quantity of the hesperidin was mixed separately with 20 mL of different solvents in conical flasks with continuous shaking and placed in a sonicator bath for 1 hour at 25°C. The sample solutions were filtered and, after appropriate dilutions, were characterized by a UV-visible spectrophotometer at 283 nm [18-20].

2.3. Drug excipients compatibility studies.

2.3.1. Fourier-transform infrared spectroscopy.

The drug excipients compatibility studies were performed on physical mixtures of hesperidin and the excipients (1:1 ratio) on Perkin Elmer Spectrum IR version 10.6.2 spectrophotometer [30].

2.3.2. Differential scanning calorimetry.

Differential scanning calorimetry (DSC) of both the hesperidin and the optimized formulation was performed on a DSC instrument (Mettler Toledo equipped with a DSC 25 cell). Weighed samples (4-10 mg, Mettler MX5 microbalance) were scanned in aluminum pans pierced with a perforated lid at 10°C min-1 in the - 50 to 350°C temperature range, under static air.

2.4. Formulation studies.

2.4.1. Preparation of Ocimum mucilage.

An appropriate amount of Ocimum seeds (20 g) were added to a specific volume of water (50 ml) at the desired temperature. The mixture was then homogenized for 6-8 hours at a constant temperature. After the complete mucilage extraction from seeds, the mucilage was extracted from seeds on a mesh screen using a rubber spatula. The obtained slurry then passed through 10 mesh screens. Separated mucilage then dried at 80 °C for 8-10 hrs on the water bath. Then the adhered mucilage was extracted by rubbing them over a 40-mesh screen. After that, the dried mucilage was weighed and recorded.

2.4.2. Preparation of hesperidin solid dispersion.

Hesperidin solid dispersion formulations (F1-F5) in different drug to carrier weight ratios were prepared by the hot-melt extrusion method. In this method, the polymer/ carrier was first melted (molten state), and then the drug was incorporated into it. The melted mixture of drug and polymer was cooled rapidly. The final mass, which was solidified after cooling, was crushed, pulverized, and sifted through mesh number 60 and stored in a desiccator [31, 32]. The compositions of prepared formulations are depicted in Table 1.

Table 1. Composition of the prepared formulations.

Formulation batches	**Ingredients (mg)**		
	Hesperidin	**Ocimum mucilage**	**Mannitol**
F1	100	30	70
F2	100	40	60

Formulation batches	Ingredients (mg)		
	Hesperidin	Ocimum mucilage	Mannitol
F3	100	50	50
F4	100	60	40
F5	100	70	30

2.4.3. Characterization of solid dispersions of hesperidin.

Solid dispersions were analyzed through micrometric evaluation, including the tapped density, bulk density, angle of repose, Carr's compressibility index, and Hausner's ratio (HR) using standard procedures [33, 34]. All the determinations were carried out in triplicate.

2.5. Determination of drug content.

The percentage drug content of formulated solid dispersion was evaluated using powder equivalent to 10 mg hesperidin and dissolved in methanol, and volume was made up to 10mL. Further, 1 mL of this stock solution was taken and suitably diluted. The solution was then filtered using Whatman filter paper and analyzed for percent drug content through a UV double beam spectrometer at 283 nm.

2.6. Saturated solubility analysis.

Saturation solubility analysis of hesperidin and prepared solid dispersions batches were evaluated using the shaking flask method. The excess amount of hesperidin (20 mg) or prepared solid dispersion (equivalent to 20 mg of hesperidin) was taken in 0.1 N NaOH (20 ml) and distilled water in the conical flask. The prepared samples were then placed in a vortex shaker bath for 24 h at 28°C. After shaking, these samples were removed, filtered, and analyzed by a UV-visible spectrophotometer at 283 nm.

2.7. In vitro dissolution studies.

In vitro dissolution studies were carried out using USP apparatus- II, paddle-type at 37°C ± 0.5°C and 100 rpm. An appropriate amount of pure drug and solid dispersion were weighted precisely. In addition, the dissolution tests of drug and solid dispersion were respectively performed in pH 6.8 buffer solution for 2 h. A 10 mL sample was taken from 900 mL dissolution media at a predetermined time interval, and each sample was passed through a 0.45µm filter. The drug content of it was evaluated by UV spectrophotometer at 283 nm. Also, 10 mL fresh medium was added after each sampling to maintain sink conditions. The cumulative percentages of pure drug and drug dissolved from the solid dispersion were calculated.

2.8. Drug release kinetics.

The *in-vitro* dissolution profile of hesperidin, solid dispersion formulations were further analyzed against the goodness of fit test through linear regression according to zero-order, 1st order, Higuchi's, Korsmeyer-Peppas, and Hixson-Crowell models to observe the drug release mechanism.

3. Results and Discussion

3.1. Pre-formulation studies.

3.1.1. Melting point.

The melting point of hesperidin was found to be 258° to 260°C, which is agreed with the existing literature.

3.1.2. UV Spectroscopy of the drug and calibration curve preparation.

The solution of hesperidin showed an absorption maximum (λmax) at about 283 nm (Figure 1). The scanned graph was according to reported literature and hence confirmed that the obtained drug sample as hesperidin. The calibration curve was plotted between the concentration and absorbance (Figure 2). The straight-line equation was y = 0.033x + 0.013, and the regression coefficient (R2) square (0.996, which shows a good correlation between concentration and absorbance.

3.1.3. Solubility of hesperidin in different solvents.

The solubility of hesperidin was determined in four different solvents (including distilled water, 0.1 N NaOH, ethanol, and 0.1 N HCl) and depicted in Table 2.

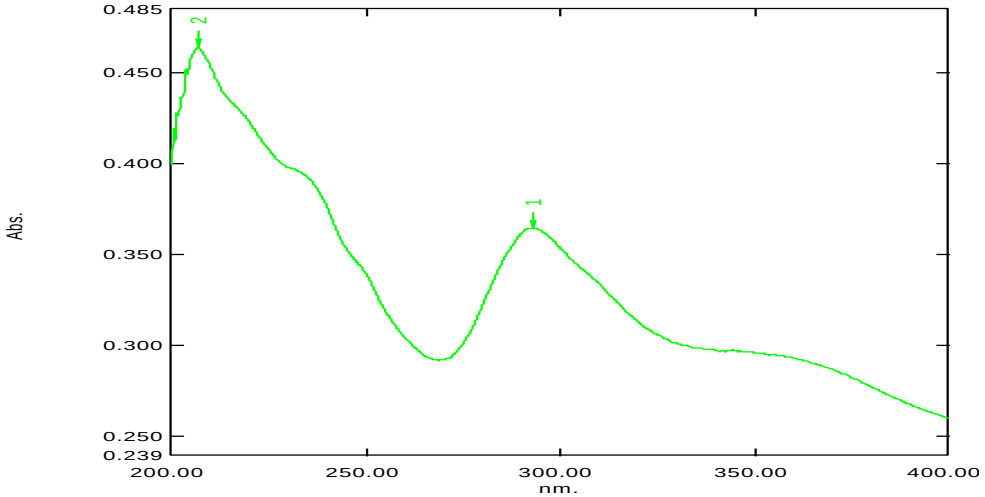

Figure 1. Absorption maximum (λmax) of hesperidin.

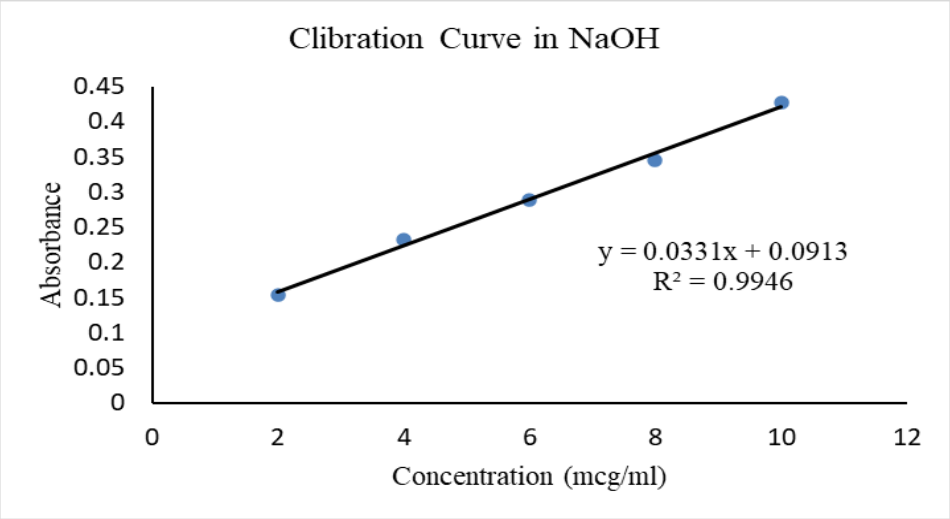

Figure 2. Calibration curve of hesperidin in 0.1 N NaOH.

Table 2. Solubility of hesperidin in different solvents.

S. No.	Solvent	Solubility in mg/mL	Inference
1	Distilled Water	3.32	Slightly soluble
2	Ethanol	23.74	Sparingly soluble
3	0.1 N NaOH	63.5	Soluble
4	0.1 N HCl	3.67	Slightly soluble

3.2. Drug-excipient compatibility study.

3.2.1. FTIR analysis.

The spectra of hesperidin represent the characteristic bands at 3300 cm^{-1} (O-H stretching); 2950 cm^{-1} (aromatic C-H stretching); 1250 and 1150 cm^{-1} (C-O stretching), respectively (Figure 3). FTIR spectra of Ocimum mucilage and hesperidin mixture did not show any noticeable shift/variation in their characteristic absorption bands, representing no chemical interaction between drug and Ocimum mucilage during the process (Figure 4).

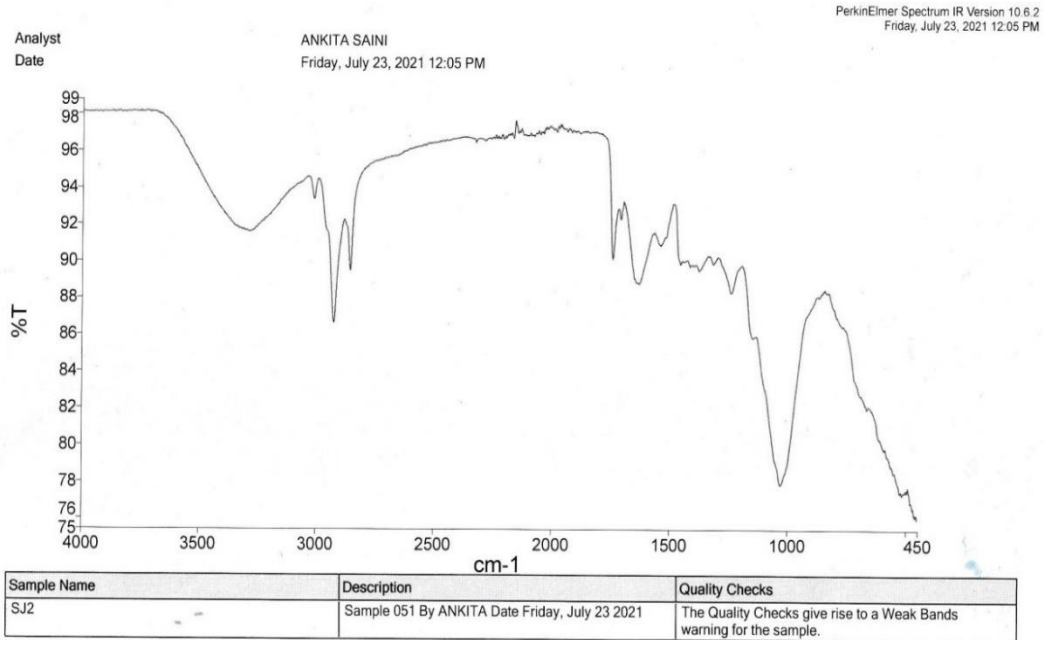

Figure 3. FTIR spectra of hesperidin.

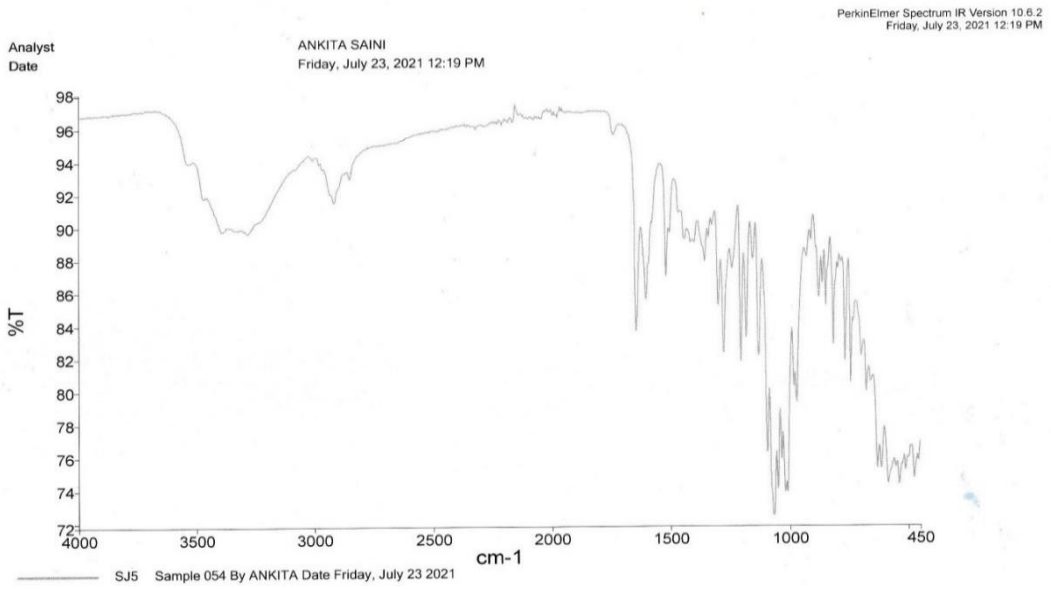

Figure 4. FTIR spectra of hesperidin and ocimum mucilage.

3.2.2. Differential scanning calorimetry.

Hesperidin showed a melting endotherm at 262.30°C that corresponds to its melting point (Figure 5).

3.3. Formulation studies.

3.3.1. Preparation of hesperidin solid dispersion.

The solid dispersions of hesperidin were successfully prepared by the hot-melt extrusion method using Ocimum mucilage and mannitol in various ratios.

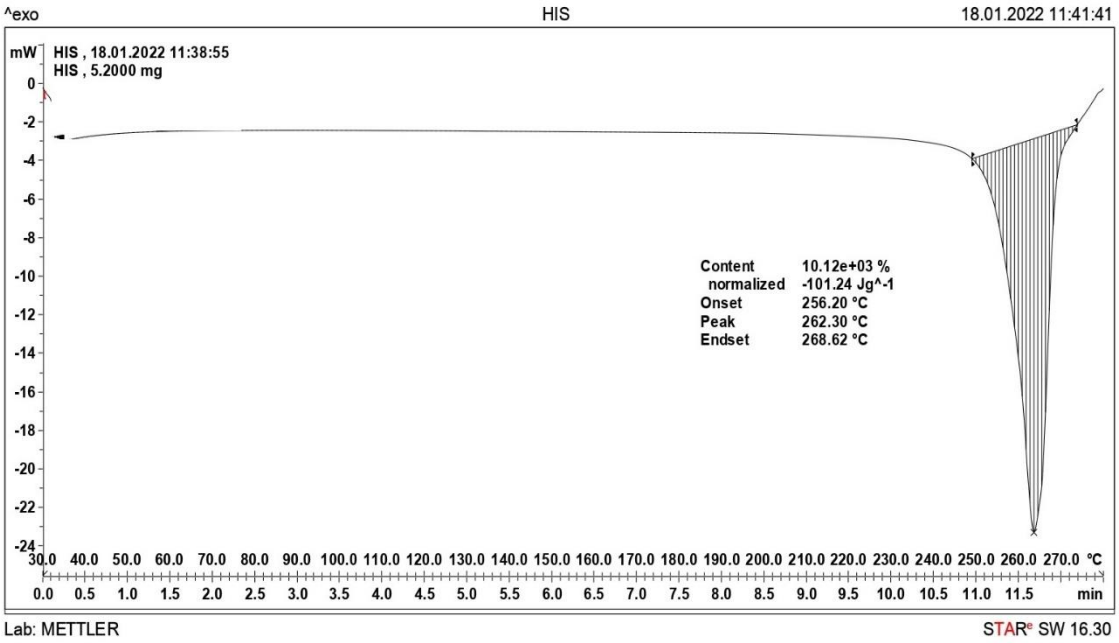

Figure 5. DSC of hesperidin.

3.3.2. Characterization of solid dispersions of hesperidin.

Micrometric characteristics like tapped density, bulk density, angle of repose, Carr's compressibility index, and Hausner's ratio (HR) showed better flow behavior of prepared solid dispersions than pure drug. The values of all these parameters are illustrated in Table 3.

Table 3. Micromeritics parameters of solid dispersion formulations.

Sr. No.	Batch Code	% Practical Yield	Hausner's ratio	Compressibility index (%)	Angle of repose (θ)
1	F1	87.5	1.18	16.11	22.68
2	F2	87.5	1.09	8.75	23.17
3	F3	91.5	1.28	22.30	24.03
4	F4	94	1.19	18.12	24.74
5	F5	92.1	1.21	16.47	26.10
6	Pure	--	1.26	24.24	24.83

3.4. Drug content.

The percentage of drug content in each preparation of solid dispersion was found to be between 87.5% to 94%.

3.5. Saturated solubility studies.

The solubility of pure hesperidin and prepared solid dispersion batches (F1- F5) in distilled water and 0.1 N NaOH is represented in Table 4.

Table 4. Solubility of pure hesperidin and solid dispersion formulations.

Formulation batch	Solubility in distilled water (mg/mL)	Solubility in 0.1 N NaOH (mg/mL)
Pure drug	0.017	0.029
F1	0.119	0.131
F2	0.185	0.198
F3	0.252	0.270
F4	**0.328**	**0.362**
F5	0.258	0.291

3.6. Characterization of optimized solid dispersion formulation.

The optimized solid dispersion formulation was further characterized by FTIR and DSC analysis. The FTIR showed no interaction between drug and polymer as characteristic peaks of hesperidin and polymers retained themselves, as shown in Figure 6.

In addition, the DSC scan of solid dispersion showed an endothermic peak at 131.06°C. The disappearance of the thermal peak of the drug indicated that the drug in solid dispersion was in amorphous form (Figure 7).

3.7. In vitro dissolution studies.

In vitro dissolution study of the prepared solid dispersion formulation (F1-F5) was carried out in 0.1 N NaOH. The %age drug release data of all the prepared formulations are presented in Table 5 and Figure 8. The %age drug release studies were carried out in triplicate.

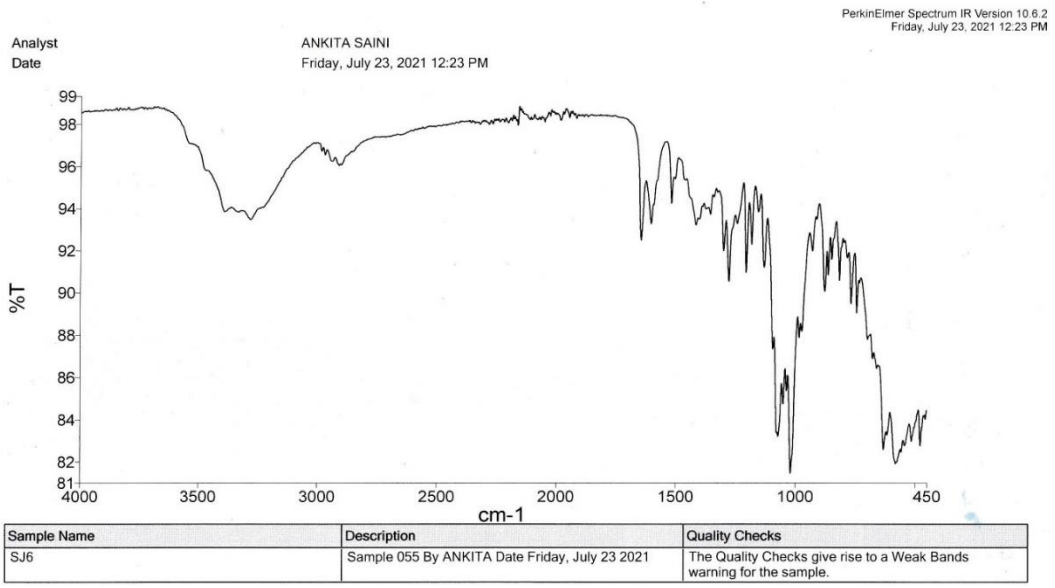

Figure 6. FTIR spectra of solid dispersion formulation F4.

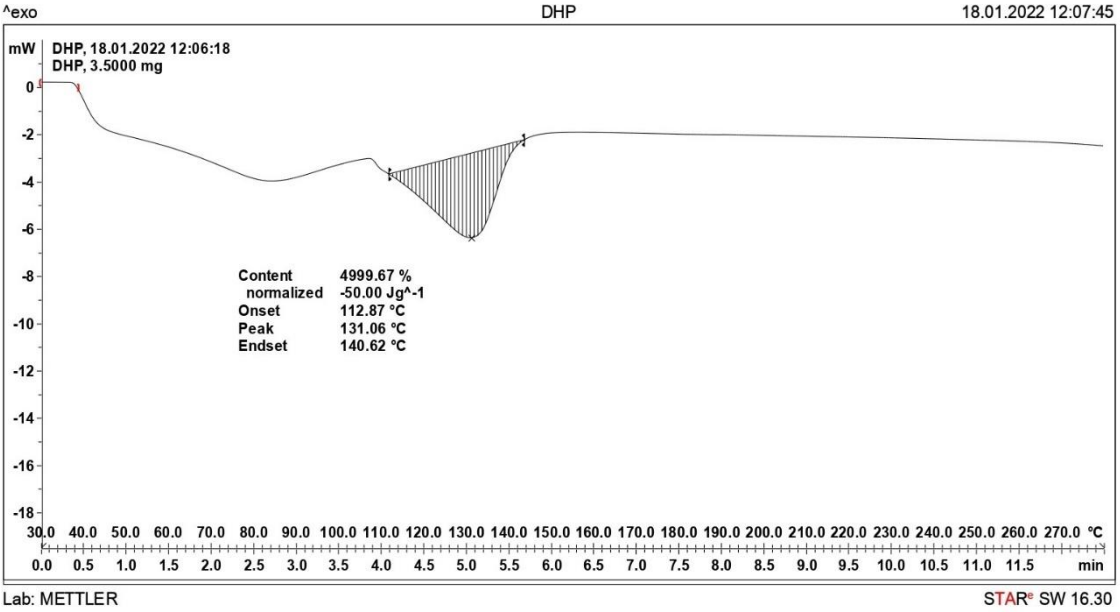

Figure 7. DSC of solid dispersion formulation F4.

Table 5. Dissolution data of pure drug in 0.1 N NaOH.

Time (in Minutes)	F1	F2	F3	F4	F5
0	0.00	0.00	0	0.00	0
5	26.45±0.02	28.64±0.04	38.73±0.08	38.73±0.04	50.18±0.01
10	30.84±0.01	31.68±0.01	43.93±0.03	44.36±0.01	54.83±0.04
15	32.79±0.04	34.17±0.07	45.62±0.07	46.11±0.06	68.51±0.05
20	36.50±0.02	39.24±0.06	46.18±0.04	46.69±0.08	79.71±0.02
30	39.13±0.06	41.61±0.08	46.87±0.01	47.38±0.03	82.15±0.07
45	41.07±0.03	46.28±0.02	56.70±0.07	57.22±0.05	88.58±0.06
60	46.27±0.02	49.74±0.09	67.31±0.05	67.94±0.07	95.75±0.08

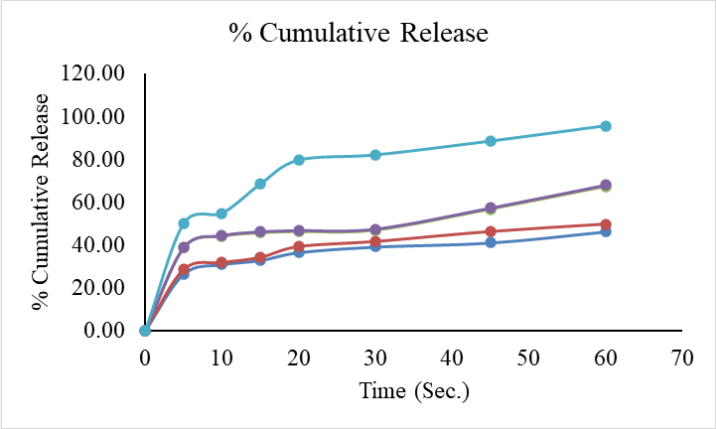

Figure 8. *In- vitro* % cumulative drug release studies of prepared formulations (F1-F5).

The dissolution of the drug was increased with an increase in the carrier ratio in the formulations. However, the dissolution of the drug was decreased in formulation F1, F2, and F3, compared to the formulations F4 and F5. From the result obtained, it can be seen that in 0.1 N NaOH, solid dispersion (batch F4), the percentage release was found 99.54 (Table 5).

3.8. Drug release kinetic mechanism.

The release data of the best formulation were fitted into five different mathematical models, i.e., zero-order, first-order, Higuchi, Korsmeyer Peppas, and Hixson-Crowell, to

characterize the mechanism of drug release. The *in-vitro* release kinetics plots of optimized formulation (F4) of hesperidin are represented in Figure 9.

The correlation coefficients (R^2) were used to indicate the best fit for each of the models considered. The *in-vitro* drug release of optimized formulation was best explained by Higuchi release kinetics because the best linearity was found in the Higuchi model equation plot (R^2 = 0.919). The mechanism followed for the drug release from the solid dispersion of hesperidin was determined by the diffusion of the drug from the insoluble matrix, as shown in Table 6.

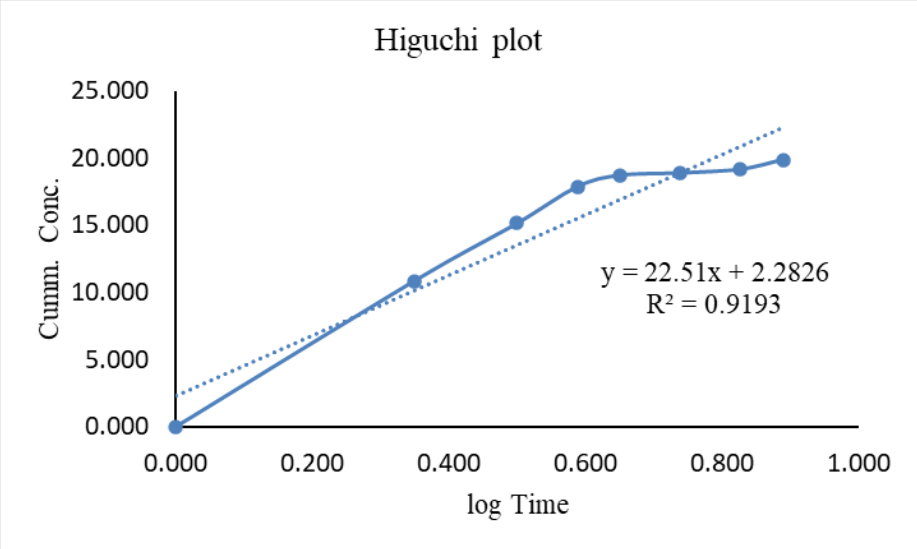

Figure 9. Higuchi plot of solid dispersion.

Table 6. Kinetic treatment of dissolution data of hesperidin solid dispersions formulation F4.

Zero Order model		First Order model		Higuchi model		Korsmeyer Peppas		Hixson Crowell	
R^2	K_0	R^2	K_1	R^2	K_H	R^2	N	R^2	K_{HC}
R² = 0.4854	y = 0.2278x + 9.7957	R² = 0.3203	y = 0.0121x + 0.7946	R² = 0.9193	y = 22.51x + 2.2826	R² = 0.8008	y = 0.0425x + 0.0207	R² = 0.3513	y = -0.0909x - 0.8264

4. Conclusions

The present study aimed to formulate solid dispersion of hesperidin using natural polymers to enhance the water solubility of hesperidin. The solubility of hesperidin was found high in basic medium (0.1N NaOH) and lower in acidic (0.1N HCl). The solid dispersion of hesperidin was prepared in Ocimum mucilage and mannitol by the hot-melt extrusion method and was characterized for drug content. Out of the five prepared solid dispersion formulations, F4 showed a marked increase in solubility compared to pure hesperidin. The FTIR confirmed no interaction between drug and polymer as characteristic peaks of hesperidin and polymers retained themselves. The *in-vitro* drug release kinetic studies were best explained by the Higuchi model with an R^2 value of 0.919, and the mechanism followed for the drug release from the solid dispersion was determined by the diffusion of the drug from the insoluble matrix. The enhanced solubility of solid dispersion of hesperidin is promising for food industry applications.

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It was declared to be none.

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Conflicts of Interest

The authors declare no conflicts of interest.

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