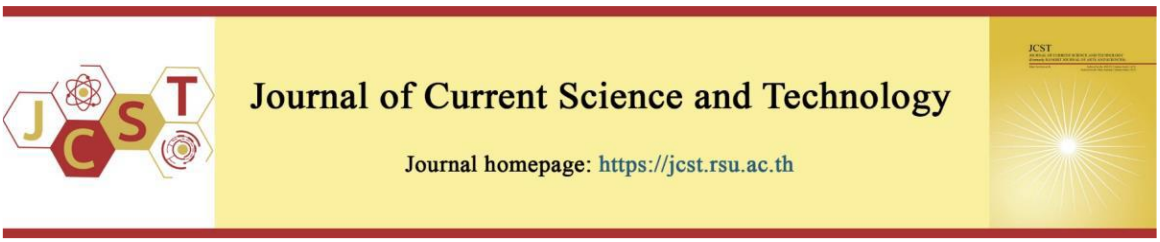
Journal of Current Science and Technology, April - June 2025 Vol. 15 No. 2, Article 104

ISSN 2630-0656 (Online)

Cite this article: Rathi, R., Sharma, A., Mujwar, S., Singh, I., Sangnim, T., & Huanbutta, K. (2025). Optimizing diabetes prediction: an evaluation of machine learning models through strategic feature selection. *Journal of Current Science and Technology*, *15*(2), Article 104. https://doi.org/10.59796/jcst.V15N2.2025.104



Development of Spray-Dried Co-crystals of Piperine and Succinic Acid for Solubility Enhancement

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Received 18 October 2024; Revised 17 December 2024; Accepted 8 January 2025; Published online 25 March 2025

Abstract

Piperine (PIP) is an amide alkaloid that belongs to the biopharmaceutical classification system II and shows poor aqueous solubility, which limits its therapeutic efficacy. To counter this issue, the objective of the current research was to screen and prepare co-crystals of piperine using succinic acid as a co-former in different molar ratios. Since spray drying is a well-known scale-up technology for co-crystallization, it has been used in the current research for co-crystal preparation. This study aimed at enhancing solubility and dissolution rate by preparing piperine co-crystals. Equimolar ratios of piperine and succinic acid were used to prepare co-crystals via spray drying. The developed spray-dried co-crystals were characterized by *in silico* modeling, solubility studies, *in vitro* dissolution studies, Powder X-ray Diffraction (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). PXRD studies of spray-dried co-crystals showed improved crystallinity, SEM studies revealed a distinct morphology compared to parent components, and the DSC thermogram indicated enhanced thermal stability. The solubility of piperine at pH 7.4 (19.2 ± 1.23 µg/mL) and pH 1.2 (35.2 ± 0.16 µg/mL) increased by almost 2-3 times which was 49.5 ± 0.89 µg/mL at pH 7.4 and 96.4 ± 1.62 µg/mL at pH 1.2. Increasing the concentration of succinic acid improves the solubility of PIP. Moreover, compared to pure piperine, the dissolution rate of piperine co-crystals increased by almost threefold. This increase may be due to the strong hydrogen bonding of piperine and succinic acid in co-crystals. The stability of piperine was also improved as the co-crystals remained stable under accelerated temperature and humidity conditions. Our findings conclude that prepared spray-dried co-crystals have the potential to improve the solubility and dissolution profile of piperine. Hence, co-crystals can be a suitable approach for improving the physicochemical properties of BCS Class II drugs.

Keywords**:** *co-crystals, piperine, succinic acid, spray drying, solubility, dissolution, stability*

1. Introduction

Piperine, also known as the king of spices (Sinha, & Ray, 2021) is a bioactive alkaloid obtained from the fruits of P*iper nigrum* and P*iper longum*. It acts as a secondary metabolite that belongs to the P*iperaceae* family. It has been widely used for its therapeutic effects including anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, hepatoprotective, antimetastatic, and antitumor (Ashokkumar et al., 2021). Research reports suggest that the limited therapeutic efficacy of drugs is primarily due to poor solubility. As a result, the therapeutic application of piperine is also limited due to its poor aqueous solubility. Thus, poorly soluble drugs affect the bioavailability and dissolution rate, inhibiting absorption in the gastrointestinal tract

(Milenković, & Stanojević, 2021; Rathi et al., 2022a). To overcome these challenges, researchers have focused on the development of many new approaches such as films, micelles, nanoparticles, bio-emulsions, micro-emulsions, and liposomes (Pentak, 2016) for drug delivery. However, poor water solubility remains an ongoing challenge for pharmaceutical manufacturers (Rathi, & Singh, 2022; Bin et al., 2020). Many researchers suggest that co-crystallization can improve the physicochemical properties of a drug without affecting its therapeutic potential (Choudhary et al., 2012; Rathi et al., 2024).

Co-crystals are multicomponent crystal systems consisting of active pharmaceutical ingredients and co-formers in the same crystal lattice held together by noncovalent interactions such as hydrogen bonding (Rathi et al., 2022b). Co-crystals modify the physicochemical properties of the drug, but the intrinsic activity remains preserved. Currently, pharmaceutical co-crystals have been listed as an encouraging approach for improving the poor solubility (Ganesh et al., 2015) and stability of drugs (Yu et al., 2020; Zaini et al., 2020). Studies have shown that Class II drugs exhibit improved solubility and physicochemical stability compared to their amorphous form (Burapapadh et al., 2024; Mo et al., 2024)

The current study on piperine-succinic acid co-crystals has achieved substantial advances in enhancing piperine solubility and dissolution rate. However, limitations still exist, such as the need for co-former screening, additional scale-up experiments or techniques, extensive stability evaluations, and in-depth mechanistic knowledge (Thenmozhi, & Yoo, 2017). With this in mind, an attempt was made to prepare spray-dried co-crystals of piperine and succinic acid to enhance solubility and dissolution rates. The co-former screening was based on *in silico* computational modelling approach. The co-crystals were also screened for evaluating physicochemical properties, stability and biological activities as well via different computational approaches. The co-crystals were prepared using the spray drying method. The obtained spray-dried co-crystals were further characterized using PXRD, DSC, FTIR, and SEM studies and evaluated for their solubility, drug content, dissolution, and stability.

2. Objective

The objective of the current research was the preparation of spray dried co-crystals of piperine and succinic acid using a spray drying technique for solubility enhancement.

3. Materials and Methods

3.1 Materials

Piperine, disodium hydrogen phosphate, potassium dihydrogen phosphate, and sodium chloride were purchased from Sigma Aldrich Chemicals Pvt. Ltd, Bangalore, India. Succinic acid was procured from Qualigens Fine Chemicals, Mumbai, India. Hydrochloric acid was purchased from Avantor Performance India Limited, Maharashtra, India. All the reagents used in this research were of high purity and commercially available.

3.2 *In Silico* Studies

3.2.1 Selection of Suitable Co-former

The 3D structure of CBZ and its co-formers (Tartaric acid, Succinic acid, Malonic acid, Ascorbic acid, and Adipic acid) were downloaded in Structure Data File (SDF) format from PubChem (pubchem. ncbi.nlm.nih.gov). All SDF files of molecules were minimized and converted into MOL2 format using Chimera 1.15 software. The files were then opened in AutoDOCK Tools 1.5.7 software, water molecules were removed, polar hydrogens were added, and the files were converted to PDBQT format. PDBQT files are protein data bank (PDB) files with provided partial charge (Q) and torsions (T). AutoDock software uses the Lamarckian genetic (LG) algorithm as a principal algorithm for conformational research by generating a trail population of diverse conformations of the ligand with mutational conformations and exchange of different parameters for consecutive generations of biological evolutions for the ultimate selection of the individuals having minimum binding energy. The additional feature of the "Lamarckian" aspect includes the individual and selective conformational search for their local conformational space followed by the identifying local minima. Later, the generated information is transferred to the later generations for predicting the binding energy of the ligand by allowing the integration of the intra-molecular energies by evaluating the energetics for their bound and unbound states on the basis of a comprehensive thermodynamic model (Mujwar, & Pardasani, 2015). The parameter required for the docking of the ligand molecule were saved in a file named docking parameter file Docking was performed five times for each conformer, and the binding affinity (Ei, kcal/mol) was recorded.

3.2.2 Biological Activity Prediction

The therapeutic potential of the piperine and succinic acid was predicted by using the Prediction of Activity Spectra for Substances (PASS) Online

webserver (Kawsar et al., 2022). PASS prediction webserver predicts the probable biological activities associated with a newer compound out of over 9,000 activity types reported in its database based upon the structural features of that particular compound based upon their Structure-Activity Relationship (SAR). Uses Quantitative Structure-Activity Relationship (QSAR) and machine learning algorithms to analyze structural features. The chemical structures of the concerned ligands were provided in MOL format as input to the webserver. The PASS online webserver evaluates the structural features of the submitted structure by concerning the available database to predict the probable activities for the submitted ligand (Mujwar, & Pardasani, 2023).

3.2.3 Stability Analysis

The thermodynamic stability for the proposed co-crystals of piperine and succinic acid was evaluated based on the chemical interactions between them by performing molecular dynamic (MD) simulation by using Schrodinger's Desmond software for a time period of 10 ns (Jain et al., 2025). The two-dimensional structure of the piperine and succinic acid was initially drawn on ChemDraw 12.0 (Cousins, 2011) and were converted to their energy-minimized three-dimensional conformation by using MM2 forcefield of Chem3D tool. The energy-minimized structures of piperine and succinic acid were docked using the Autodock tool to obtain the co-crystallized chimera of piperine-succinic acid which was further utilized to analyze the stability of the proposed chimera by using MD simulation (Valdés-Tresanco et al., 2020). MD simulation was executed by using TIP3P explicit water model to create an orthorhombic-shaped simulation box with a 10 Å gap between the wall of the box and the complex. The simulation box's is-osmotic environment was created by adding counter ions with 0.15 M NaCl to neutralize the existing charge. The system's energy was minimized by running 2000 iterations using a 1 kcal/mol merging threshold. Using an energy-minimized complex system, 100 ns of MD simulation was executed. A steady 300 K temperature with 1.013 bars of pressure was maintained throughout the simulation process. To construct simulation interaction graphs after the simulation process, the trajectory path has been defined as 9.6 with the energy interval of 1.2 ps (Shinu et al., 2022).

3.2.4 Physicochemical Analysis

The key physicochemical parameters of the piperine and succinic acid, utilized for preparation of co-crystals were evaluated for their pharmacokinetic profile using the SwissADME webserver. The physicochemical properties of a specific ligand are purely dependent on their structural features and are having a huge impact on the smooth movement of drugs along the human body (Shah, K., & Mujwar, S. 2022).

3.3 Preparation of Co-crystals

The PIP-SA co-crystals were successfully synthesized via the spray drying method. The equimolar ratios of PIP and SA were weighed and dissolved in ethanol with different ratios. The prepared solvent was spray dried using Laboratory Spray Dyer (LSD-40, Jay Instruments and Systems Pvt Ltd., India). The process parameters of spray drying were as follows: Inlet temperatures were maintained between 80-100°C with a flow rate of approximately 900-1000gm/h. The powder was further dried under a vacuum (Li et al., 2020; He et al., 2017; Patil et al., 2014). Various batches of co-crystals were prepared by varying the concentration of drug (PIP): co-former (SA) and coded as F1 (1:1), F2 (1:2), F3 (2:1), and F4 (1:10) (Hibbard et al., 2023)

3.4 Drug Content

An accurately weighed amount of co-crystals, equivalent to 10 mg of piperine, was dissolved in 10 mL of buffer solution. Then further samples were filtered using Whatman filter paper (Grade 41) and the solution was diluted appropriately and analyzed for drug content at 345nm using a UV-visible spectrophotometer (Systronics, 2202, Ahmedabad, India).

3.5 Solubility Study

The solubility studies were performed at room temperature using an orbital shaker of REMI, C-24 BL, India at two distinct pH levels i.e., 7.4 and 1.2. Co-crystals containing 10 mg of piperine were mixed in a 100 mL medium and equilibrated. At the end of 24 hours, the resulting clear solution was filtered for removal of solid residual, if any. The concentration of piperine was calculated by using a UV spectrophotometer at 345 nm.

3.6 Fourier Transform-Infrared Spectroscopy (FT-IR)

The physiochemical interactions of pure piperine and its co-crystals were determined using FT-IR analysis. The FTIR spectrophotometer of Alpha Bruker, IFS 66/S, Germany was employed for FTIR

studies (Garbacz, & Wesolowski, 2018). The dry potassium bromide was mixed with samples in the ratio 1:100 and compressed to form pellets. The samples were scanned within the range of 4000 cm^{-1} to 600 cm^{-1} wavenumbers with a resolution of 2 cm^{-1}.

3.7 Powder X-ray Diffraction Analysis (PXRD)

The PXRD analysis was performed on Panalytical, Netherlands, PW 30/40 X-ray diffractometer, using Cu K ∝ radiation generated at 45 kV and 40 mA for pure piperine and its PIP-SA co-crystals components. The data were recorded within the range of 2θ = 5º to 40º.

3.8 Scanning Electron Microscopy (SEM)

The morphological studies of piperine, PIP-SA co-crystals were examined using scanning electron microscope, Jeol-JSM 6510 LV, Japan. 10 mg powdered sample was weighed and placed over a specimen stub of aluminum having double sided adhesive carbon tape and coated with gold for approximately 5 seconds making use of sputter coater SCD 005 (BAL – TEC) for 100 s at 30 mV.

3.9 Differential Scanning Calorimetry (DSC)

DSC was employed for identifying the thermal behavior of piperine and PIP-SA co-crystals using DSC-60N, Japan. A 5 mg sample was mounted over an aluminum pan and compressed using a hydraulic press with a continuous heating rate of 10ºC/min from 40 to 200 ℃

3.10 *In Vitro* Dissolution Study

The dissolution studies were performed for pure piperine and PIP-SA co-crystals using Dissolution Apparatus Type II (Lab India, DS8000 Pune, India), operated at 50 rpm for 2 hours dissolution testing. Phosphate buffer (pH 7.4) and 0.1N HCl (pH 1.2) were used as dissolution media at 37 ± 0.5℃. An equivalent weight of co-crystals containing an excess amount of drug was dispersed in 900 mL of dissolution media. An aliquot of 5 mL of all samples was collected at predetermined times and replaced with fresh dissolution media each time to maintain the sink conditions. The collected samples were filtered and analyzed using a UV spectrophotometer at 345 nm.

3.11 Stability Study

Stability studies were performed to evaluate the considerable changes that can occur during the storage of co-crystals. Pure piperine and PIP-SA co-crystals were kept in a stability chamber in triplicate at 40℃/75% RH for 3 months. The stability of the prepared formulations was evaluated by assessing their optical properties and *in vitro* drug release.

3.12 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA). A p-value of < 0.05 was used to indicate statistical significance in all cases. Data were analyzed as ± mean standard deviation.

4. Results

4.1 *In Silico* Studies

4.1.1 Selection of Suitable Co-former

Table 1 shows the results of the piperine virtual screening with different co-former. One of the most methods in structure-based drug design is molecular docking, which predicts the binding conformation of small-molecule ligands to their target binding sites. By making a grid box with the dimensions, the docking site on the molecular target was found. The optimal conformation with the lowest docked energy was selected following the completion of the docking search. The affinity of a molecule is inversely proportional to its binding strength; lower affinity values indicate stronger binding. In the present study, succinic acid showed the lowest affinity (-2.6 kcal) and indicating the strongest binding as compared to other co-formers.

Table 1 *In-silico* screening of co-former with Piperine.

Co-former	Affinity (kcal)
Succinic acid	-2.6
Tartaric acid	-1.2
Ascorbic acid	-1.5
Adipic acid	-1.5
Malonic acid	-1.7

4.1.2 Biological Activity Prediction

Based on its input structural properties, the PASS online web server forecasts the ligand's probability of activity (Pa) and inactivity (Pi). In order to find similar structural properties in the available therapeutic compounds, the webserver looks for the structural features in the submitted ligand in the database that has been made available (Daina et al., 2017). The likelihood that a compound possesses similar therapeutic potential is determined by the structural similarities between the submitted ligand and known medicinal drugs. Table 2 lists the outcomes of the evaluation of the piperine and succinic acid utilizing the PASS online web server. The PASS online

software's output showed a number of PIP and SA's pharmacological characteristics. The two components did not share any pharmacological characteristics. Since the two components exhibit distinct biological characteristics, their combination may enhance the drug's therapeutic effectiveness.

4.1.3 Stability analysis

MD simulation of the piperine-succinic acid chimera for 10 ns by embedding it within the semipermeable membrane model demonstrated that the chimera remains thermodynamically stable throughout the simulation (Mujwar, 2021). The thermodynamic stability of the proposed co-crystallized chimera for a nanosecond timescale range suggests that the chimera can move efficiently across different compartments of the human body and potentially mitigate delayed absorption and reducing piperine-induced gastric toxicity. The observed chemical interaction between the piperine and succinic acid in the proposed chimera is shown in Figure 1.

Table 2 The probability of activity as well as the probability of inactivity of the prepared co-crystals predicted by PASS online web server

Activity	Probability of Activity (Pa)	Probability of Inactivity (Pi)
Piperine		
Membrane integrity agonist	0.916	0.007
Carminative	0.826	0.003
Neurotransmitter uptake inhibitor	0.814	0.004
Sigma receptor agonist	0.734	0.004
MAP kinase stimulant	0.663	0.009
Antidyskinetic	0.648	0.027
Ovulation inhibitor	0.627	0.013
Antiulcerative	0.609	0.010
Caspase 3 stimulant	0.575	0.019
Succinic Acid		
Acylcarnitine hydrolase inhibitor	0.961	0.002
Alkenylglycerophosphocholine hydrolase inhibitor	0.950	0.003
Alkylacetylglycerophosphatase inhibitor	0.948	0.002
Phobic disorders treatment	0.949	0.003
Methylamine-glutamate N-methyltransferase inhibitor	0.946	0.001
CYP2J substrate	0.946	0.002
Acrocylindropepsin inhibitor	0.945	0.003
Chymosin inhibitor	0.945	0.003
Acylcarnitine hydrolase inhibitor	0.945	0.003

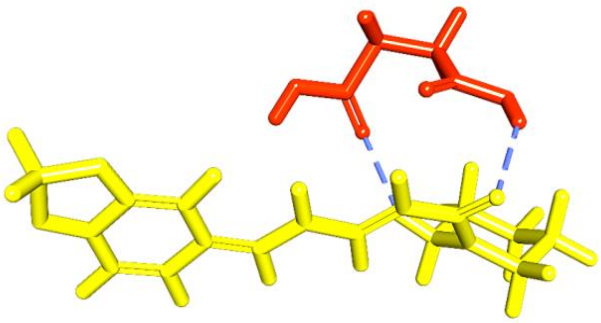

Figure 1 chemical structure of piperine and succinic acid co-crystals prepared by using ChemDraw software

Table 3 Physicochemical properties of the co-crystallized chimera of piperine with succinic acid obtained by using SwissADME webserver

Molecule	MW	Heavy atoms	Rot. Bonds	HBA	HBD	TPSA	cLogP	BBB permeant	Pgp substrate	log Kp (cm/s)	Lipinski #violation	Veber #violations	Bio availability Score
Piperine	285	21	4	3	0	38.77	3.41	Yes	No	-5.58	0	0	0.55
Succinic Acid	118	8	3	4	2	74.60	-0.63	No	No	-7.44	0	0	0.85

Table 4 Drug content uniformity of prepared co-crystals

Formulation code	Drug: co-former ratio	% Drug content (mean ± SD)
F1	1:1	68.54 ± 0.7
F2	1:2	67.38 ± 0.5
F3	2:1	67.72 ± 0.4
F4	1:10	70.33 ± 0.8

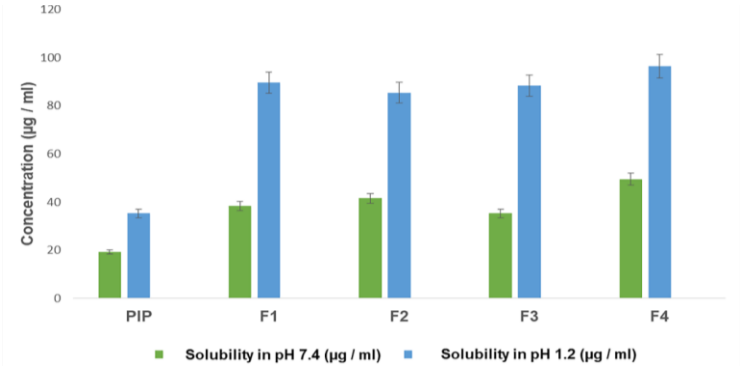

Figure 2 Solubility studies of pure PIP and PIP-SA co-crystals (F1, F2, F3, and F4) in pH 1.2 and 7.4

4.1.4 Physicochemical Analysis

The results obtained after analysis of the physicochemical properties of piperine, and succinic acid by using the SwissADME webserver were tabulated in Table 3. Both piperine and succinic acid, the proposed ligands for co-crystal formation, exhibit excellent physicochemical properties in accordance with Lipinski's Rule of Five, including molecular weight, TPSA, cLogP, HBD, and HBA. Both molecules possess all evaluated properties within the prescribed range, with no violations of Lipinski's or Veber's rules. Thus, the resulting piperine-succinic acid co-crystals may effectively address piperine's poor bioavailability and gastric toxicity.

4.2 Drug Content

The drug content is necessary for confirming improved product development. The drug content of the prepared co-crystals ranged from 67.38 ± 0.5% to 70.33 ± 0.8%. The drug content was higher for F2 and F4 co-crystals as mentioned in Table 4. This may be attributed to the higher succinic acid content relative to the drug.

4.3 Solubility Study

The solubility of pure piperine was found to be 19.2 ± 1.23 µg/mL at pH 7.4 and 35.2 ± 0.16 µg/mL at pH 1.2. The co-crystals enhanced PIP solubility, with F4 exhibiting the highest solubility: 49.5 ± 0.89 µg/mL at pH 7.4 and 96.4 ± 1.62 µg/mL at pH 1.2, as represented in Figure 2. As per our research findings, increasing succinic acid concentration increases piperine's solubility. The possible reason for this might be the microenvironmental solubilizing properties of succinic acid impacting the wettability of piperine in an aqueous medium resulting in solubility enhancement. On the other hand, the solubility increases in the pH but more at pH 1.2, this could be due to the hydrogen ions in an acidic medium which accelerates the dissolution of the drug. This suggests that co-crystals rapidly dissolve in the gastric environment, leading to enhanced gastric absorption of piperine (Fitriani et al., 2022; Ullah et al., 2015).

4.4 Fourier Transform Infrared Spectroscopy (FT-IR) analysis

The FT-IR spectra of piperine and spray-dried PIP-SA co-crystals were performed to study the solid-state interaction between the molecules. The comparative FTIR spectra of piperine and different PIP-SA co-crystals are shown in Figure 3. Piperine showed characteristics peaks at 1490 cm^{-1} (weak C=C), 1586 cm^{-1} (N-O bonding), 2710 cm^{-1}, 2858 cm^{-1} (C-H bonding), 3204 cm^{-1} (N-H-stretching) and 3323 cm^{-1} resembling C-H stretching. As per the reported literature, the oxygen from the ketone group of PIP and hydrogen of succinic acid is mainly involved in the formation of hydrogen bonds (Ullah et al., 2016). In the case of PIP-SA co-crystals, the peaks at 2938 cm^{-1} and 2651 cm^{-1} represent the O-H stretching. The IR spectra showed distinct peaks of pure piperine but no characteristic peaks confirmed the chemical interaction between pure drug and conformer (Octavia et al., 2023; Al-Dulaimi et al., 2022).

4.5 Powder X-ray Diffraction (PXRD) analysis

The PXRD diffractograms for piperine and their multielement co-crystals (F1 and F4) are shown in Figure 4. The descriptive peaks of piperine were found at 2θ value *viz.* 14.95°, 19.93°, 22.53°, 25.90°, and 28.43°, which corresponds to the existing literature (Liu et al., 2022). The PXRD pattern of spray-dried PIP–SA co-crystals appeared to be different from pure piperine and a greater number of peaks were observed at 2 θ = 13.75°, 20.38°, 22.24°, 24.56°, 28.00° that indicates the formation of a new crystalline form. As per the literature study, the co-crystal peak determines the quantitative amount of co-crystalline phase present in samples as compared to pure drugs (Ober, & Gupta, 2012). The enhanced crystallinity may be attributed to the presence of succinic acid in co-crystals, which increases the ordering of crystal lattice. This study reveals that a higher-ordered crystal lattice was the reason behind the high crystallinity of co-crystals as compared to pure piperine.

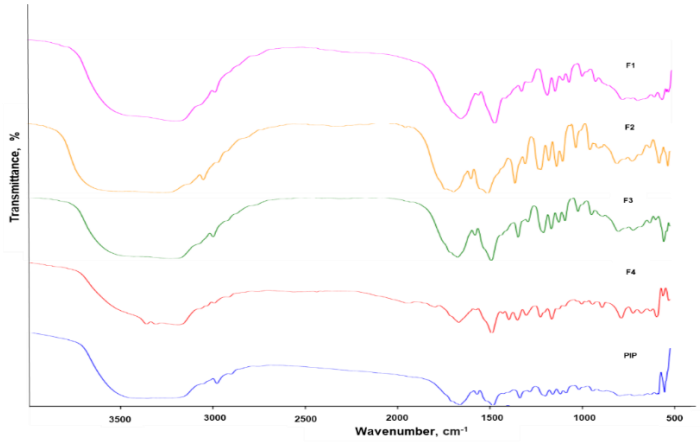

Figure 3 FT-IR spectra of pure PIP and PIP-SA co-crystals (F1, F2, F3 and F4).

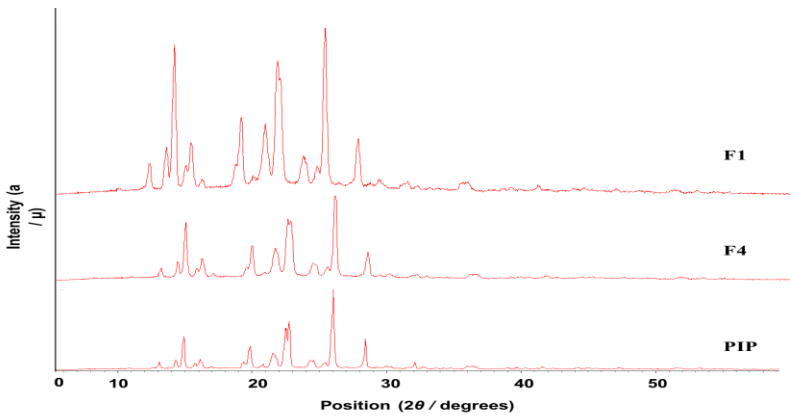

Figure 4 PXRD pattern of PIP and PIP-SA co-crystal (F1 and F4).

4.6 Scanning Electron Microscopy (SEM) Analysis

SEM was utilized for studying the morphological characteristics of piperine and PIP-SA co-crystals. The morphologies obtained of piperine, F1, and F4 are shown in Figure 5. The piperine showed uneven rosewood-like structures. Whereas co-crystal morphology represented fluffy rosette and rock-shaped structures along with semi-spherical shaped particles. However, a significant morphological change was observed with F4. The difference in morphology of PIP and PI-SA co-crystals might be due to the dispersion of succinic acid on piperine with flattened radial agglomeration around a focal point. Similar sort of results was obtained by the research study of Itraconazole and succinic acid co-crystals and the irregularly shaped flakes were also obtained which were due to initial flake nucleation (Wicaksono et al., 2019). The difference in morphological characteristics was due to the interaction between piperine and succinic acid molecules, which modifies the crystal faces of pure PIP and therefore crystal morphology.

4.7 Differential Scanning Calorimetry (DSC) analysis

The DSC was employed to study thermal behavior, most importantly the melting temperatures of the powder samples. The DSC thermogram of piperine showed a sharp endothermic peak at 131°C, corresponding to its melting point, as shown in Figure 6. The sharp endothermic peak at 158.5°C was recorded for F4 whereas F1 depicts peaks at 185.48°C. The higher melting point of co-crystals could be indicative of stronger intermolecular interactions between piperine and succinic acid, indicating higher stability, physical properties, and reduced hygroscopicity. F4 were selected for further studies.

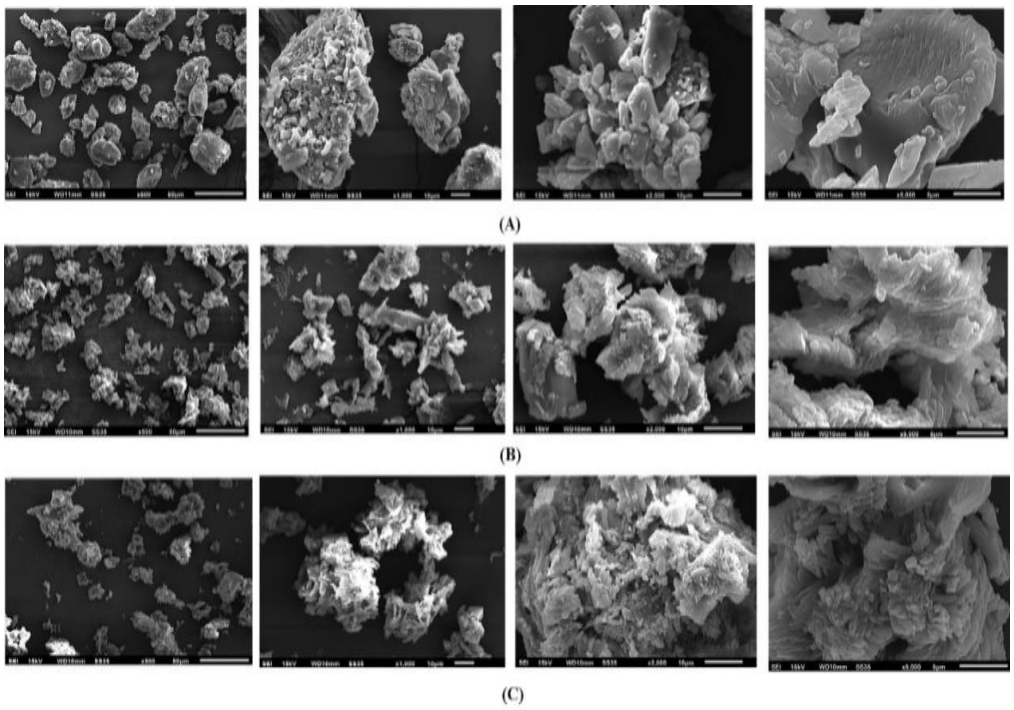

Figure 5 SEM images of (A) Pure PIP (B) F2, and (C) F4 at 500x, 1000x, 2000x and 5000x.

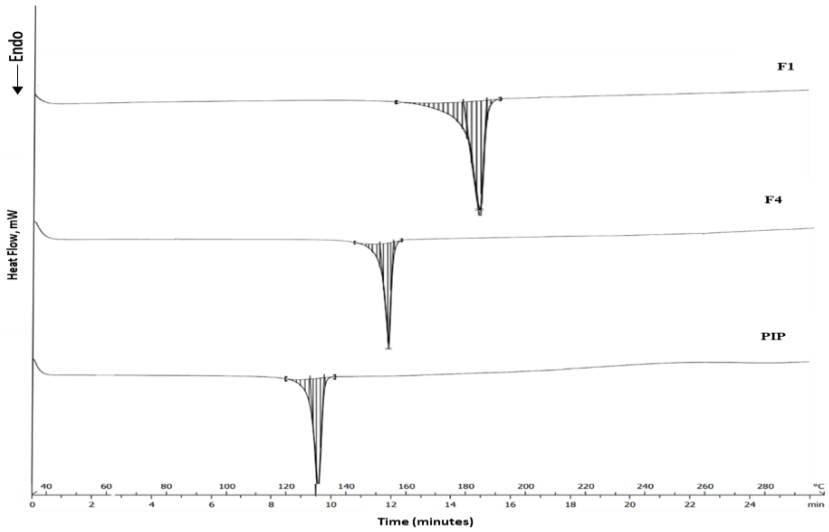

Figure 6 DSC plots pure PIP and PIP-SA co-crystals.

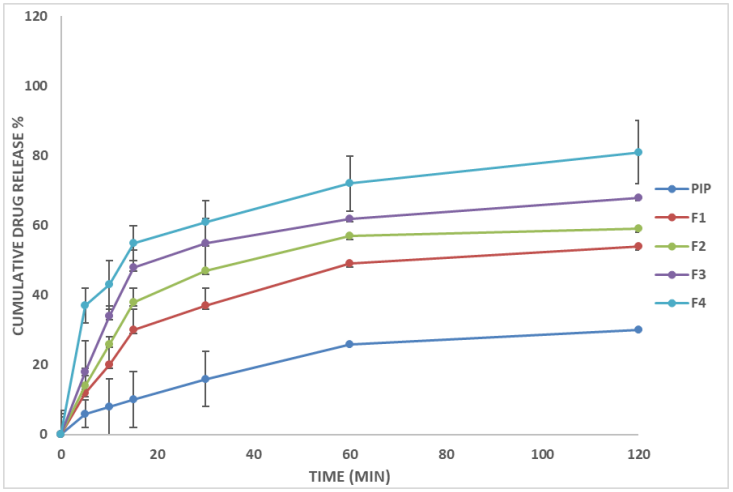

Figure 7 Cumulative release of drug (%) versus time (min) graph represents dissolution profile of piperine and (PIP-SA) co-crystals in phosphate buffer, pH 7.4.

4.8 *In vitro* Dissolution Study

According to the Noyes-Whitney equation, the solubility and dissolution of a solid are proportional (Hasanah et al., 2023). In the current research, as the solubility of co-crystals was higher, the dissolution for PIP–SA co-crystals was also higher at both pH 7.4 and pH 1.2, as depicted in Figures 7 and 8. After 2 hours of dissolution studies, the PIP release was only 30.02 ± 0.02% (pH 7.4) and 25.07 ± 3.02% (pH 1.2). In contrast, the co-crystals showed a faster dissolution profile as compared to pure piperine. This change in the dissolution profile of piperine suggests that co-crystallization alters the crystal structure of piperine during co-crystallization (Pentak, 2016). Out of all co-crystals the maximum drug release was observed in the case of pH 1.2 which was 81.68 ± 0.71% (F3) and 99.01 ± 2.37% (F4). This shows approximately 3-fold enhanced drug release from co-crystals in acidic conditions. Regarding solubility profiles, co-crystals showed a similar dissolution pattern depicting rapid drug release in acidic conditions. This also indicates the formation of immediate drug-release dosage forms. It might be possible that strong hydrogen bonding of PIP-SA co-crystals in which formation of channel structure initiated by succinic acid, solubility enhancement delivered through succinic acid co-crystals could be a highlight factor in dissolution study.

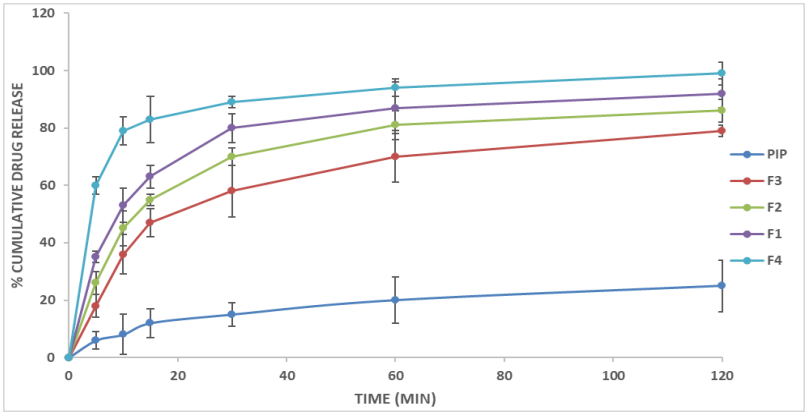

Figure 8 Cumulative release of drug (%) versus time (min) graph represents dissolution profile of piperine and (PIP-SA) co-crystals in 0.1N HCl, pH 1.2

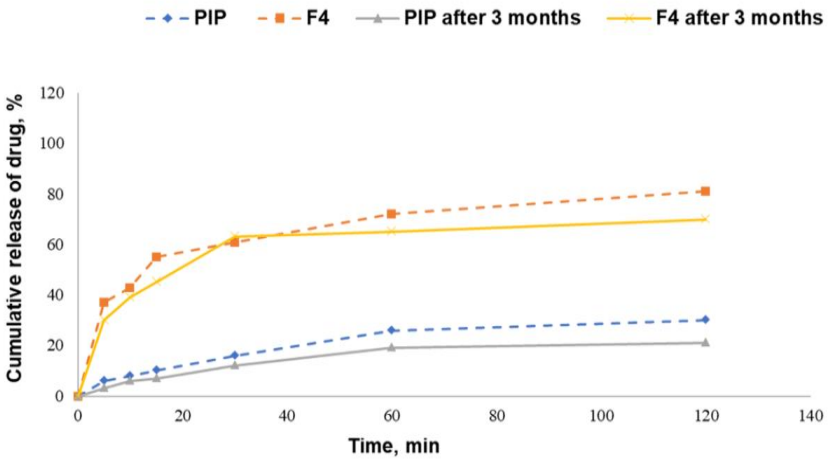

Figure 9 Cumulative release of drug (%) versus time (min) graph represents stability studies of PIP and F4 formulation for 3 months in phosphate buffer, pH 7.4

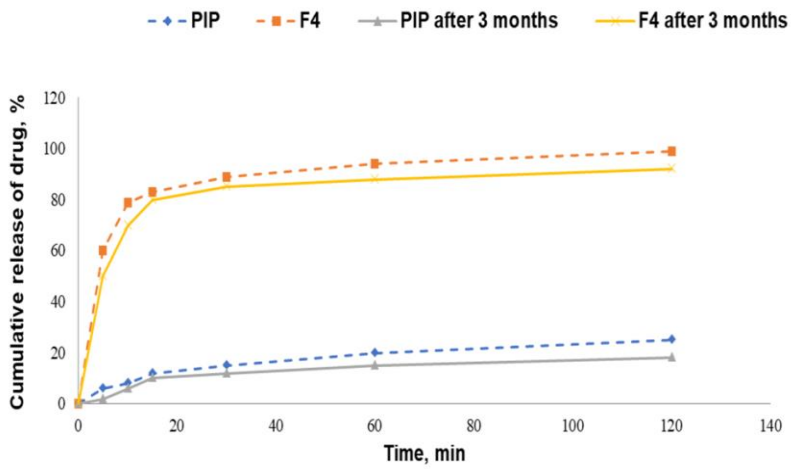

Figure 10 Cumulative release of drug (%) versus time (min) graph represents stability studies of PIP and F4 formulation for 3 months in 0.1N HCl, pH 1.2.

4.9 Stability Study

Stability studies were executed to evaluate the considerable changes that can occur during the storage of co-crystals. Pure piperine and PIP-SA co-crystals (F4) were kept in a stability chamber for 3 months in triplicate at 40°C/75% RH. The stability of formulations was assessed based on optical properties and *in vitro* drug release. Piperine and F4 were kept in a stability chamber for 3 months at 40° C/75% RH and checked for their appearance and *in vitro* drug release after 2 hours. The co-crystals were found to be the same after the release studies (Jain et al., 2017) and their physical appearance has not changed at all and they showed negligible change in the drug release characteristics of co-crystals. The change that appeared *in vitro* drug release is shown in Figures 9 and 10.

5. Discussion

This study aimed to synthesize piperine-succinic acid co-crystals. The computational studies identified succinic acid as a suitable co-crystal former candidate and highlighted the co-crystal stability and excellent physicochemical properties. PXRD, DSC, and SEM analysis confirmed the formation of co-crystals. SEM studies confirmed the formation of a new crystalline phase with morphological properties distinct from parent components. The identified PXRD diffractograms for co-crystals revealed higher crystallinity as more peaks and peak intensity were observed. The DSC thermogram of piperine exhibited a sharp endothermic peak at 131.94°C whereas co-crystals demonstrated intensified single endothermic peaks at 185.85°C (F1) and 155°C (F4), indicating higher intermolecular interactions, stability, reduced hygroscopicity, and lower melting. The co-crystals exhibited a drug content of approximately 67-70%, a desirable property in formulation development.

Solubility and *in vitro* dissolution profiles were evaluated at pH 7.4 and pH 1.2. An increase in solubility of PIP was observed at the pH but the maximum was at pH 1.2, which might be due to the hydrogen ions in acidic medium which accelerates the drug dissolution. The dissolution rate of co-crystals increased approximately 3-fold compared to that of pure piperine. The improved solubility and more than 80% drug release after two hours of dissolution studies suggest the development of an immediate drug-release dosage form. Zaini et al. (2020) described the mechanism wherein the highly soluble co-former molecules dissolve first from the co-crystal, leading to the formation of a void channel structure. As the strong intermolecular interaction between the API and the conformer weakens, the stability of the co-crystal decreases. Thus, the remaining stacked API structure with just weaker intermolecular connections would simply disassemble. As a result, API molecules dissolve, enhancing the active pharmaceutical ingredient's solubility. In this study, the strong inter molecular interaction was the hydrogen bonds between piperine and succinic acid in the co-crystal; the succinic acid co-former formed the channel structure. Thus, this solubility improvement mechanism can be applied to the succinic acid co-crystal. Furthermore, increasing the concentration of succinic acid improved solubility and dissolution rates. Stability studies showed no significant changes in the physical appearance or drug-release characteristics of the co-crystals.

6. Conclusion

In this study, we successfully prepared spray-dried piperine-succinic acid co-crystals. The prepared PIP-SA co-crystals showed improved solubility and dissolution rate. The FT-IR, DSC, and PXRD confirmed the formation of co-crystals. In future studies, these formulations can be adapted to develop tablets or capsules with enhanced bioavailability. Other than just succinic acid different co-former and other dicarboxylic acid combinations can be selected for co-crystal formation that possess the anti-inflammatory properties of piperine. The study can also be extended to studying different process parameters as well such as effect of temperature (inlet temperature, outlet temperature) on the physicochemical properties of drugs and co-crystals. Additionally, quantitative analysis of particle size distribution and its effect on solubility and dissolution can be explored. These findings encourage future in vivo studies to evaluate piperine's potential for clinical applications. So, it can be useful in developing different routes of administration too.

7. Acknowledgments

The authors are thankful to Chitkara College of Pharmacy, Chitkara University, Punjab, India for support and institutional facilities.

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