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Original Article

PREPARATION, CHARACTERISATION, EVALUATION AND DFT ANALYSIS OF CILNIDIPINE-L-PHENYLALANINE COCRYSTAL

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ABSTRACT

Objective: The objective of this study was to prepare, characterise and evaluate pharmaceutical cocrystals of Cilnidipine using L-phenylalanine as the coformer to enhance the aqueous solubility of Cilnidipine. It was also proposed to study the mechanism of cocrystal formation based on Density Functional Theory (DFT) using Gaussian software.

Methods: To overcome the limitation of poor aqueous solubility of Cilnidipine, a 1:1 pharmaceutical cocrystal of Cilnidipine was prepared using L-phenylalanine as the coformer by liquid assisted grinding (LAG) technique. The resultant cocrystals were characterised by Fourier transform-infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and field emission scanning electron microscopy (FE-SEM). They were evaluated for their saturation solubility in water. The mechanism of cocrystal formation was studied at the DFT level of theory.

Results: The band broadening of the–NH and–NO peaks in FTIR spectra of Cilnidipine indicated the formation of hydrogen bonds in the prepared cocrystals. A single sharp melting endotherm at 218.40 °C in the DSC curve confirmed the formation of cocrystals. The appearance of new peaks in the PXRD pattern of the prepared cocrystals showed the formation of a new crystalline phase. FE-SEM analysis also confirmed the above findings. The prepared cocrystals exhibited 3.31 folds enhancement in saturation solubility. The DFT analysis showed the formation of intrmolecular hydrogen bonding between the–NO of Cilnidipine and–NH of L-phenylalanine.

Conclusion: The present study demonstrated a successful approach for enhancing the solubility of poorly water-soluble drug Cilnidipine by cocrystallisation technique using L-phenylalanine as the coformer.

Keywords: Cocrystals, Cilnidipine, L-phenylalanine, Coformer, Liquid assisted grinding, Solubility enhancement

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INTRODUCTION

Solid dosage forms such as tablets and capsules are highly preferred and widely accepted oral drug delivery systems as they offer the advantage of enhanced patient compliance with minimal discomfort [1, 2]. The successful delivery of low-soluble drugs through the oral route is limited by its poor oral bioavailability and is a significant challenge for researchers to develop a suitable formulation of such drugs. Various strategies are adopted to improve the physicochemical properties. In recent years, the cocrystallisation technique has gained significant importance due to its ability to improve the physicochemical properties of active pharmaceutical ingredients (API), such as solubility, stability and mechanical properties, without altering their chemical and pharmacological properties [3–8]. Pharmaceutical co-crystals are multi-component crystalline materials consisting of two or more different molecules, with one of them being the API and the other being a cocrystal former (co-formers), present in a defined stoichiometric ratio within the same crystal lattice and are associated with nonionic and non-covalent bonds [9, 10].

Uncontrolled hypertension is a significant risk factor for cardiovascular, renal and endocrine disorders. Long-acting dihydropyridine calcium channel blockers (CCBs) are used as first-line agents for hypertension, either as a monotherapy or in combination with other antihypertensive agents [11, 12]. Cilnidipine is a unique and effective 1, 4 dihydropyridine CCB, which blocks both L and N-type calcium channels. Cilnidipine is the preferred medication for treating diabetic patients with hypertension due to its reported reno-protective, cardio-protective, and antioxidant activity [13-16]. Cilnidipine is a BCS class II drug with poor aqueous solubility, leading to decreased bioavailability of less than 13%. Cilnidipine is a very weak acid with a pKa value of 11.39. Cilnidipine has one hydrogen bond donor and eight hydrogen bond acceptors and can form supramolecular synthons (N-H--N, N-H--O, N-O--H-) with suitable coformers [17–20]. Hence, the aim of the study was to prepare, characterise and evaluate the cocrystals of Cilnidipine using the liquid-assisted grinding (LAG) method. L-phenylalanine, an amino acid, is used as the coformer because it's amino and carboxylic acid functional groups can act as both hydrogen bond donors and acceptors and tend to form hydrogen bonds with various hydrogen bonding sites of Cilnidipine [21–23]. Additionally, L-phenylalanine is considered safe for human use and is listed in the SCOGS database (Select Committee on GRAS: Generally Regarded as Safe Substances) [24, 25]. Furthermore, the ΔpKa value of the Cilnidipine-L-phenylalanine system is less than one, and therefore, the probability of cocrystal formation is high [26, 27].

In this paper, pharmaceutical cocrystals of Cilnidipine-L-phenylalanine were prepared by the LAG method, a simple, efficient, cost-effective, reliable and green method for cocrystal discovery. The obtained cocrystals were characterised by Fourier transform-infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), and field emission scanning electron microscopy (FE-SEM). The saturation solubility studies of the prepared cocrystals were carried out to evaluate the change in the aqueous solubility of the cocrystal formed. The intermolecular and intramolecular interactions of the Cilnidipine-L-Phenylalanine cocrystals were studied by a computational technique based on density functional theory (DFT) using the Gaussian approach [28, 29].

MATERIALS AND METHODS

Cilnidipine was a gift sample from Pure Chem. Pvt. Ltd. Gujarat. All the chemicals used were of analytical grade. L-phenylalanine was procured from Merck, and Methanol (HPLC grade) was procured from SD Fine-Chem. Limited, Mumbai. Double distilled water used for the study was prepared in the laboratory.

Preparation of cilnidipine-L-phenylalanine cocrystals by LAG method

Pharmaceutical cocrystals of Cilnidipine with L-phenylalanine as the coformer were prepared by the LAG method. The stoichiometric ratio of Cilnidipine and L-phenylalanine, as given in table 1, were weighed and manually ground in an agate mortar and pestle at room temperature with the addition of 2 drops (100 µl) of methanol at regular intervals for 45 min. A schematic representation is shown in fig. 1. The resulting products were collected and dried at room temperature in a desiccator. The obtained products were subjected to further studies.

Table 1: Ratio of cilnidipine and L-phenylalanine in the cocrystals

Formulation codes	Cilnidipine: L-phenylalanine molar ratio
CP1	1:0.5
CP2	1:1
CP3	1:2

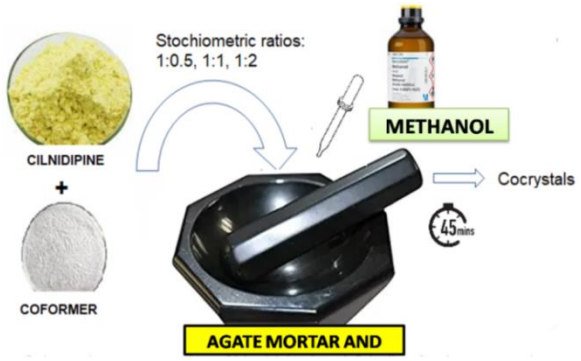

Fig. 1: Schematic representation of LAG for preparation of cilnidipine-L-phenylalanine cocrystals

Fourier transform infrared spectroscopy (FTIR)

The infrared spectra were obtained by the KBr disk technique [30], using Nicolet iS10 FTIR Spectrometer, Thermofisher, USA. The KBr disk containing Cilnidipine, L-phenylalanine, prepared cocrystals of Cilnidipine-L-Phenylalanine, and physical mixture in the same ratio as that used in the preparation of cocrystals were placed in the spectrometer and scanned in the range of 4000 to 400 cm^{-1} at spectral resolution of 0.4 cm^{-1}.

Thermal property measurements

The DSC analyses of the samples were performed in a differential scanning calorimeter, DSC Q20V24.11, TA Instruments, USA. 3 mg of sample was carefully placed and crimped in a non-hermetic aluminium pan. It was then scanned from 30 °C to 300 °C at a heating rate of 10 °C/min, under a continuously purged dry nitrogen atmosphere.

Powder X-ray diffraction (PXRD) measurements

Powder XRD patterns of all samples were recorded on an Ultima IV Diffractometer (Rigaku, Japan), using Cu-Kα X radiation as the source (λ = 1.54056 A°) at 40 kV/30 mA. Diffraction patterns were recorded over the 2θ range of 5°–80° at a step size of 0.02°/0.5 s.

Saturation solubility measurements of cilnidipine-L-phenylalanine cocrystals

Excess quantities (about 100 mg) each of pure Cilnidipine and Cilnidipine-L-phenylalanine cocrystals in 10 ml water were taken in a vial to determine the saturation solubility. The vials were shaken continuously in a shaker water bath at 100±10 agitations/min for 24 h. The resulting solutions were then centrifuged, the supernatant separated and filtered using Whatman filter paper No: 10. The absorbance of the filtrate was measured at 242 nm using a UV spectrophotometer and the saturation solubility was determined.

Field emission scanning electron microscopy (FE–SEM)

Surface topography and morphological evaluation of Cilnidipine, L-phenylalanine and Cilnidipine-L-phenylalanine cocrystals were studied by FE–SEM using GeminiSEM 300 (Carl ZEISS, Germany). The samples were first gold sputtered under an argon atmosphere to render them conductive. Then, it was sprinkled and dispersed onto an aluminium stub surface fixed with double-sided adhesive tape. The gold-sputtered samples were analysed using an accelerating voltage of 20 kV.

Computational analysis-DFT studies

The mechanism of cocrystal formation and possible interactions in Cilnidipine–L-phenylalanine cocrystals was computationally analysed. All the quantum calculation was done based on density functional theory using the Gaussian 09 software package [31]. The input structures of Cilnidipine (PubChem CID: 5282138) and L-phenylalanine (PubChem CID: 6140) were obtained from the database of PubChem [32]. Open Babel application was used to convert these structures from SDF (standard data format) to GJF (Gaussian Job File) input files [33]. Frontier molecular orbital analysis was done to understand the molecular properties. The structures of all the molecules under study were optimised in the ground state, and calculations were done using the B3LYP/6-311++G (d,p) level of the theory [34].

RESULTS AND DISCUSSION

Preparation of cilnidipine-L-phenylalanine cocrystals

Pharmaceutical cocrystals of Cilnidipine-L-phenylalanine were prepared by mixing Cilnidipine and L-phenylalanine in molar stoichiometric ratios of 1:0.5, 1:1, 1:2 by LAG method using methanol as the solvent. Methanol acts as a catalyst that assists in cocrystal formation by improving the wettability of Cilnidipine, thus enhancing the probability of intermolecular hydrogen bonding between Cilnidipine and L-phenylalanine and thus increasing the chances of cocrystal formation [35, 36]. The obtained products were air-dried and stored in desiccators for characterisation and evaluation.

Fourier transform infrared spectroscopic measurements

Infrared spectroscopy is a reliable and frequently used spectroscopic technique to study the interaction between the API and coformer, especially in detecting the hydrogen bond formation in cocrystals [7, 37]. The IR spectra of formulation CP1 and CP3 did not show characteristic changes in the absorption frequencies from their components, suggesting the absence of cocrystal formation. The FTIR spectra of Cilnidipine (CIL), physical mixture of Cilnidipine and L-phenylalanine (CPPM] and cocrystals of Cilnidipine and L-phenylalanine in molar ratio 1:1 (CP2) are shown in fig. 2.

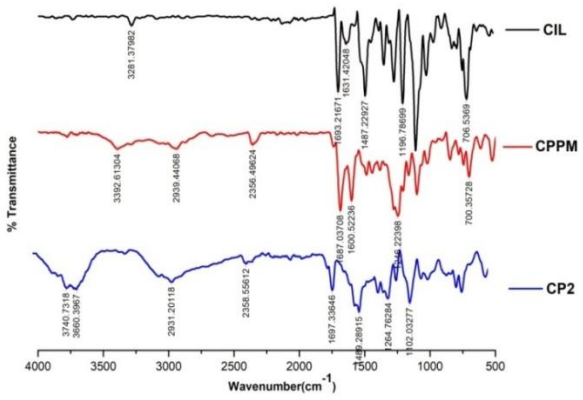

Fig. 2: FTIR spectral interpretation of cilnidipine-L-phenylalanine cocrystal and its components

Cilnidipine (CIL) gave characteristic peaks at 3281 cm^{-1}(N-H str of 1, 4 dihydropyridine), 1693 cm^{-1} (-C=O str), 1525 $cm^{-1}$and 1343 cm^{-1} (N-O sym and asym stretching), 1097 cm^{-1} (C-N-2° amine bending vibrations). The spectrum of the physical mixture did not show any characteristic change in peaks of Cilnidipine, indicating the absence of any physical or chemical interactions between them. In formulation, CP2, the typical peak of Cilnidipine at 3281 cm^{-1} (NH str) is broadened and gave an absorption band from 3800–3500 cm^{-1},indicating the possibility of hydrogen bonding between–NH of Cilnidipine and -OH group of L-phenylalanine. Band broadening is also observed in the region between 1650 cm^{-1} to 1100 cm^{-1} suggestive of formation of hydrogen bonding between NO_2 of Cilnidipine and -NH of L-phenylalanine. These features are suggestive of the formation of co-crystals [38, 39].

Thermal property measurements

The Cilnidipine (Cil), L-phenylalanine (PA), physical mixture (CPPM) and the cocrystals obtained were subjected to DSC analysis. The thermogram appeared as exothermic or endothermic peaks depending on the thermal events during the DSC analysis. The thermogram of the CP2 (fig. 3) exhibited characteristic changes and were different in pattern, indicating the formation of cocrystals.

The thermograms of Cilnidipine and L-Phenylalanine (Cil and PA in fig. 3) showed a single sharp melting endotherm at 109.69 °C and at 272.75 °C corresponding to their respective melting points, indicating that they melt without decomposing. Hence, DSC can be used to screen and characterise cocrystals formed [40]. An equimolar physical mixture of Cilnidipine and L-phenylalanine (CPPM in fig. 3) showed an endothermic peak at 106.80 °C corresponding to eutectic melting, an exothermic peak at 145.58 °C of cocrystal formation and a melting endotherm at 225.72 °C due to cocrystal melting. These three characteristic features are typical of systems capable of cocrystal formation [41–43]. The thermogram of CP2 showed a sharp endothermic peak at 218.40 °C, a temperature between the characteristic melting endotherm of Cilnidipine and L-phenylalanine. This shift in melting point from that of the respective API and coformer is due to a change in the crystal lattice of Cilnidipine in the presence of the coformer, indicating the formation of a new crystalline form of the drug [44]. The summary of thermal events of CP2 and its components is given in table 2.

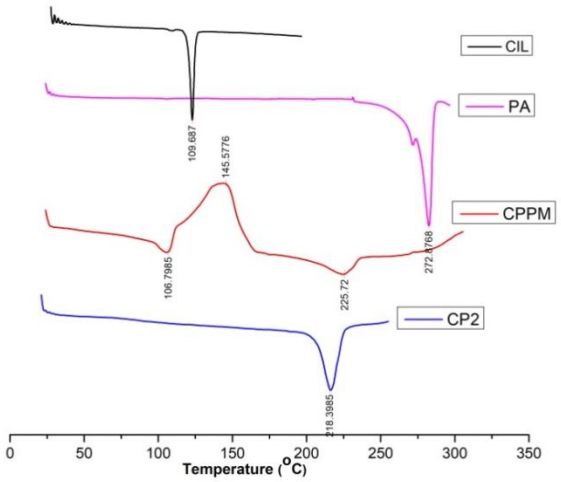

Fig. 3: Overlain DSC curves of cilnidipine (Cil), L-phenylalanine (PA), physical mixture of Cilnidpine and L-phenylalanine (CPPM), and cilnidipine-L-phenylalanine cocrystal (CP2)

Table 2: Summary of DSC analysis of cilnidipine (Cil), L-phenylalanine (PA), physical mixture (CPPM) and cocrystal (CP2)

Name of the sample analysed	Endothermic/Exothermic	Onset temperature	Peak	Heat of reaction/Enthalpy
Cilnidipine (Cil)	Endothermic	106.92 °C	109.69 °C	35.71 J/g
L-Phenylalanine (PA)	Endothermic	264.63 °C	272.75 °C	310.6 J/g
Physical Mixture (CPPM)	Endothermic	98.20 °C	106.80 °C	47.44 J/g
	Exothermic	132.58 °C	145.58 °C	-
	Endothermic	211.62 °C	225.72 °C	21.81 J/g
Cocrystal (CP2)	Endothermic	209.49 °C	218.40 °C	58. 03 J/g

Powder X-ray diffraction (PXRD) measurements

PXRD is a non-destructive technique that detects changes in the crystal lattice of a molecule. Each molecule has its own characteristic XRD pattern, and a change in the crystal lattice of a molecule alters the 2θ position of peaks and its relative intensity. A new crystalline phase gives an XRD pattern different from that of its parent molecule, and therefore, PXRD studies are helpful for the identification of a new crystalline phase [45–47]. The PXRD pattern of Cilnidipine (CIL), L-phenylalanine (PA), and the cocrystals CP2 are depicted in fig. 4. The characteristic 2θ positions of Cilnidipine, L-phenylalanine and cocrystals are given in the table 3.

Table 3: 2θ positions of cilnidipine, L-phenylalanine and CP2

Sample	Characteristic 2θ positions in °(deg)
Cilnidipine	5.86, 11.74, 12.3, 14.26, 16.32, 18.74, 19.96, 20.76, 21.8, 23.2, 23.98, 25.0, 26.12, 27.18, 28.26, 28.26 and 29.8.
L-Phenylalanine	5.52, 16.8, 22.54, 28.34 and 34.16.
CP2	5.66, 10.72, 11.72, 12.32, 14.26, 16.48, 17.72, 18.72, 19.12, 19.82, 21.78, 22.56, 23.32, 23.9, 24.92, 26.04, 27.06, 28.34 and 29.8.

In the diffraction pattern of CP2, a new peak at 17.72 and 19.12 can be seen, and there were shifts in the positions of other peaks. There was a significant increase in the intensity of the peaks in CP2 compared to Cilnidipine. Thus, CP2 displayed unique crystalline patterns that are different from Cilnidipine and L-phenylalanine, indicating the formation of a new crystalline phase.

Saturation solubility measurements of cilnidipine-L-phenylalanine cocrystals

The saturation solubility of Cilnidipine and formulation CP2 were determined. The results are shown in table 4. The cocrystal CP2 exhibited 3.31 folds enhancement in aqueous solubility compared to the pure drug. This enhancement of the aqueous solubility of CP2 may be due to the change in the crystal lattice of Cilnidipine in the presence of L-phenylalanine. The FTIR analysis of CP2 demonstrated the formation of hydrogen bonding suggestive of cocrystal formation, which was further confirmed by the DSC analysis. This change in the crystal lattice of Cilnidipine due to cocrystal formation has resulted in its enhanced aqueous solubility.

Field emission-scanning electron microscopy

FE-SEM images of Cilnidipine, L-Phenylalanine, physical mixture (CPPM) and CP2 are given in fig. 5.

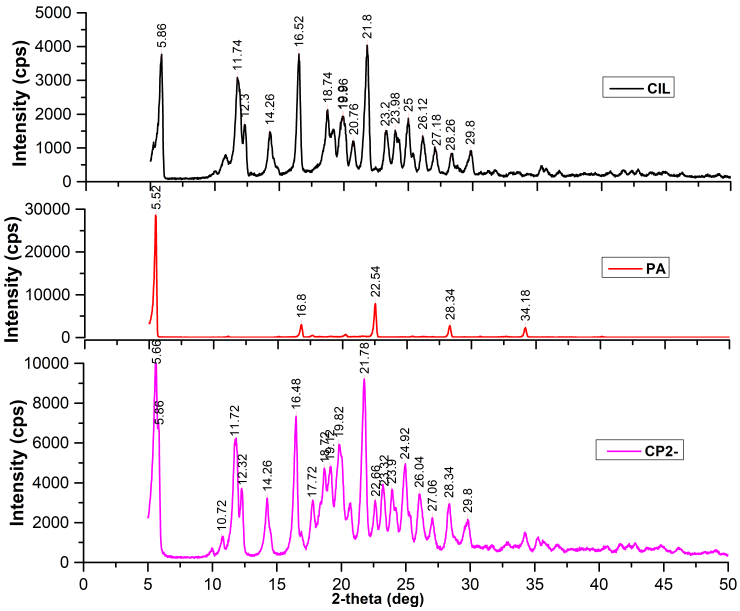

Fig. 4: PXRD pattern of cilnidipine (Cil), L-phenylalanine (PA) and cilnidipine-L-phenylalanine cocrystal (CP2)

Table 4: Saturation solubility studies of cilnidipine-L-phenylalanine cocrystals in water

Sample (n=3)	Solubility in water µg/ml	No. of folds increment
Cilnidipine	5.53±0.26	
Cilnidipine-L-phenylalanine cocrystal (CP2)	18.33±0.61	3.31

Value shown is the mean±SD (n = 3)

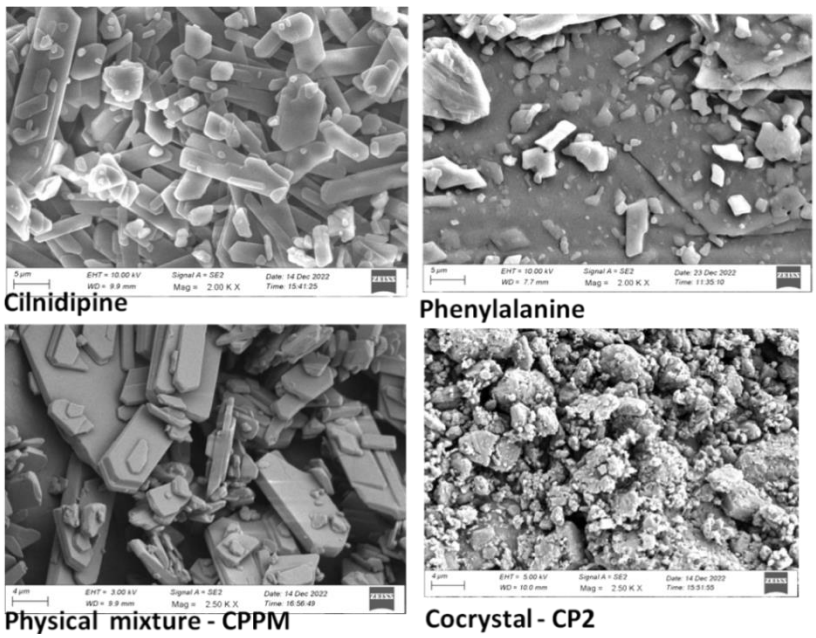

Fig. 5: SEM image of cilnidipine, L-phenylalanine (mag: 2.00 KX), physical mixture and cocrystal (CP2) (mag: 2.50 KX)

The pure Cilnidipine appeared as thin, smooth textured, rectangular flakes or sheets. L-phenylalanine was seen as irregular lumps. No characteristic changes were seen in the surface morphology of the drug or the coformer in the image of the physical mixture. In CP2, a considerable size reduction of the components is seen, and the sheet-like appearance of Cilnidipine was converted to nearly spherical morphology during the cocrystal formation by liquid-assisted grinding. The coformer L-phenylalanine was seen adsorbed onto the surface of the Cilnidipine. This reduction in the particle size, increase in surface area, and adsorption of L-phenylalanine onto the surface of hydrophobic Cilnidipine contributed to enhanced wettability and solubility of Cilnidipine.

Computational analysis-DFT studies

The optimised structures of Cilnidipine, L-phenylalanine and Cilnidipine-L-phenylalanine cocrystals are shown in fig. 6 to 8, respectively.

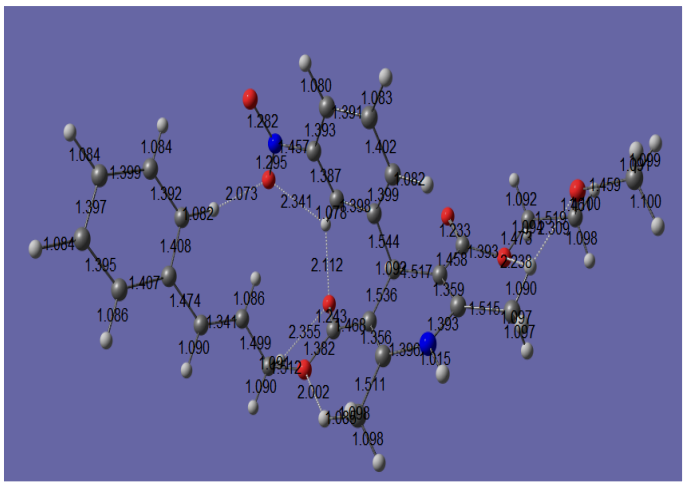

Fig. 6: Optimized structure of cilnidipine using B3LYP/6-311++G(d,p) method

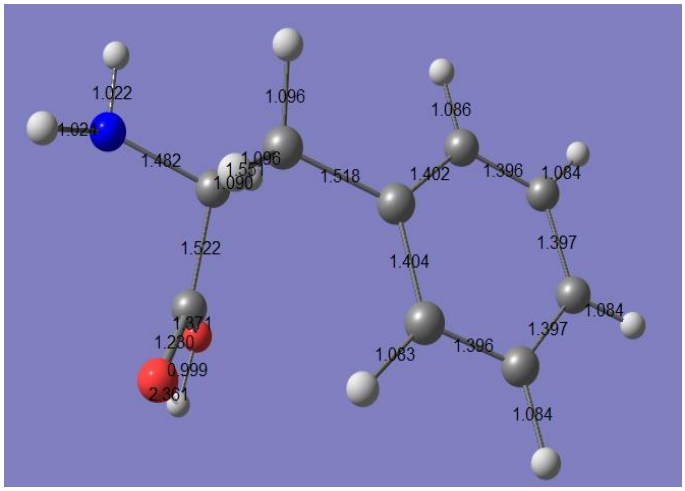

Fig. 7: Optimized structure of L-phenylalanine using B3LYP/6-311++G(d,p) method

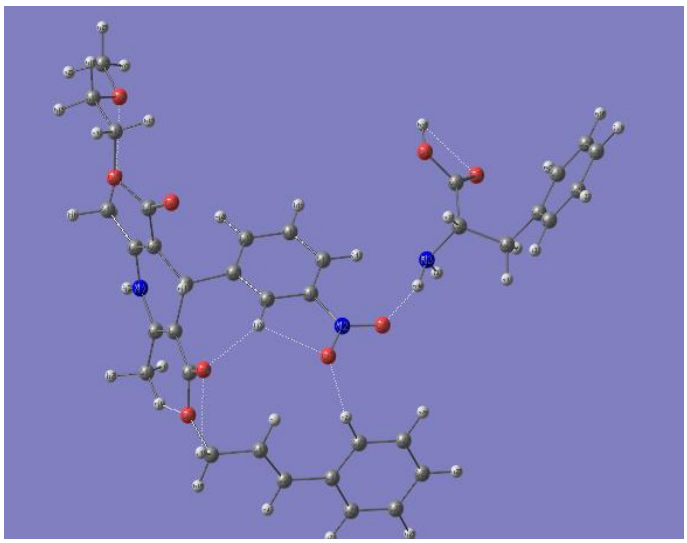

Fig. 8: Optimized structure of cilnidipine-L-phenylalanine cocrystals using B3LYP/6-311++G(d,p) method

Bond lengths, bond angles and dihedral angles of Cilnidipine, L-phenylalanine and Cilnidipine-L-phenylalanine cocrystals were calculated. The details of the intermolecular and intramolecular hydrogen bonding in these molecules are tabulated in table 5:

Table 5: Intermolecular hydrogen bonding in cilnidipine, L-phenylalanine and cilnidipine-L-phenylalanine cocrystals and their bond length

Definition	Bond length A°	Definition	Bond length A°	Definition	Bond length A°
Intramolecular hydrogen bonding in cilnidipine		**Intramolecular hydrogen bonding in L-Phenylalanine**		**Intramolecular and Intermolecular hydrogen bonding in Cilnidipine-L-phenylalanine cocrystals**	
R(1-43)	2.238	R(2-23)	2.361	R(1-43)	2.216
R(2-45)	2.002			R(2-45)	2.006
R(4-39)	2.112			R(4-39)	2.118
R(4-52)	2.355			R(4-52)	2.356
R(5-43)	2.309			R(5-43)	2.325
R(7-39)	2.341			R(6-82)	1.98
R(7-60)	2.073			R(7-39)	2.303
				R(7-60)	2.085
				R(66-87)	2.355

Significant changes in the bond length occurred due to the cocrystal formation between Cilnidipine and L-phenylalanine. An intrmolecular hydrogen bond of (bond length-1.98 A°) is formed between the oxygen of the nitro group (R6) of Cilnidipine and hydrogen of the amino group (R82) of L-phenylalanine (fig. 9).

Frontier molecular orbital analysis

Frontier molecular orbital analysis helps predict the chemical reactivity and stability of molecules. It depends on the transitions between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbit (LUMO). The energies of HOMO and LUMO represent their ability to donate and accept electrons, respectively. The HOMO-LUMO energy difference (ΔE, also called activation energy) gives the compound's band gap or energy gap. The chemical reactivity and stability of Cilnidipine-L-phenylalanine cocrystals were studied by calculating the HOMO and LUMO energies and their energy gaps (E_{LUMO}–E_{HOMO}) by using B3LYP/6-311++G (d,p) basis set and is shown in fig. 10.

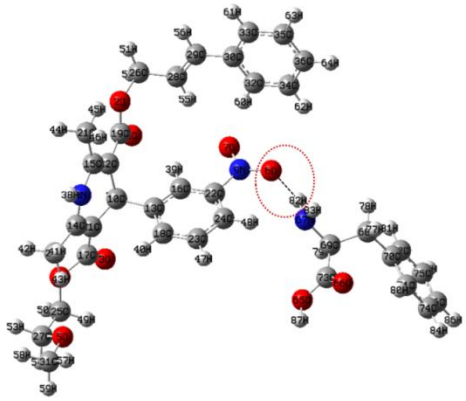

Fig. 9: Intramolecular hydrogen bonding in cilnidipine-L-phenylalanine cocrystal

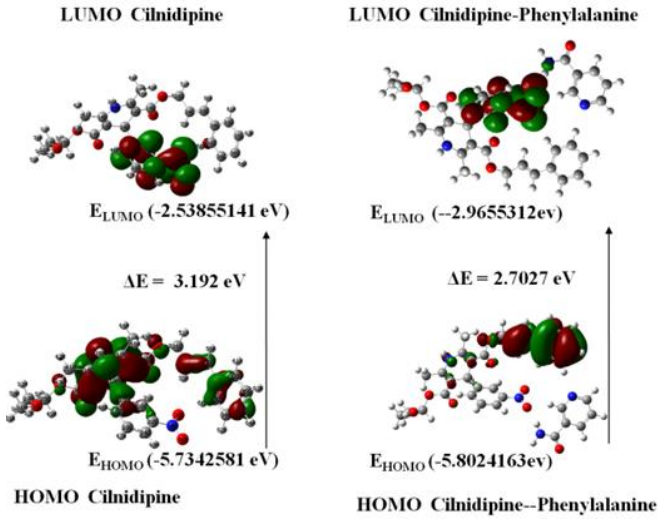

Fig. 10: HOMO–LUMO plot of the cilnidipine and cilnidipine-L-phenylalanine cocrystal

The calculated HOMO–LUMO energy gap of Cilnidipine and Cilnidipine-L-phenylalanine cocrystal are 3.192 eV and 2.7027 eV, respectively. The HOMO and LUMO orbital energy gap decreased in the Cilnidipine-L-phenylalanine cocrystal, indicating that the cocrystal is more reactive than the API [48, 49].

CONCLUSION

The cocrystallisation approach using the liquid-assisted grinding technique was adopted for the solubility enhancement of Cilnidipine with L-phenylalanine as the coformer. FTIR spectra of the formulation indicated hydrogen bonding between API and coformer. DSC and PXRD analysis confirmed the formation of a new crystalline phase. FE-SEM analysis revealed a reduction in particle size and a change in surface morphology. The cocrystal formulation exhibited a 3.31-fold enhancement in aqueous solubility compared to pure Cilnidipine. Computational analysis of cocrystals established the intramolecular hydrogen bonding between the Cilnidipine and phenylalanine. The present study demonstrated a successful approach for enhancing the solubility of poorly water-soluble drug Cilnidipine by cocrystallization technique using L-phenylalanine as the coformer.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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