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Research Article

What if Cocrystallization Fails for Neutral Molecules? Screening Offered Eutectics as Alternate Pharmaceutical Materials: Leflunomide-a Case Study

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ABSTRACT

Background: The manuscript is aimed to optimize the biopharmaceutical parameters of a poorly soluble, neutral anti-rheumatic drug 'leflunomide' by preparing its non-covalent derivatives (NCDs). For this various monocarboxylic acids- (adipic acid, picolinic acid) and dicarboxylic acids (maleic acid, malonic acid, sorbic acid), as well as pyridine carboxamide derivatives (nicotinamide, isonicotinamide), are used as coformers.
Methods: The novel solid forms were rationally prepared and systematically characterized. Further, these solid forms were subjected to equilibrium solubility and intrinsic dissolution rate (IDR) analysis in three aqueous media (pH 1.2, pH 4.5 and pH 6.8). *In vivo* plasma studies in male Wistar rats were done to assess the effect on area under the curve (AUC) and the maximum concentration (C_{max}) of leflunomide in prepared solid forms.
Results: These NCD were primarily characterized to be eutectics rather than cocrystals as expected. The stoichiometry was established by phase diagrams. The negative value of heat of mixing indicated them to be of cluster type. In addition, leflunomide in eutectics showed approximately 9 folds increase in solubility up to 4 hours. Besides this, approximately 4 folds enhancement in the in IDR was also observed. Maximum increase in bioavailability indicated by enhanced values of AUC and C_{max} (490.29 µg h^{-1} mL^{-1} and 31.42 µg mL^{-1}, respectively) for leflunomide-maleic acid eutectic in comparison to pure LEF (AUC: 193.20 µg h^{-1} mL^{-1} and C_{max}: 12.09 µg mL^{-1}).
Conclusion: The unsuccessful cocrystallization experiments were found to be the latent eutectics. The evaluation of these novel eutectics of poorly soluble drug exhibited possibility to further amplify the scope of accessible material phase options other than pure active pharmaceutical ingredient (API) without disturbing the structural integrity.

Introduction

Design of new solid forms (predisposed with hydrogen bonding functionalities) on the basis of non-covalent interactions is the cornerstone of the pharmaceutical industry to modulate the biopharmaceutical parameters of API having poor solubility and bioavailability.[1,2] For this purpose cocrystallization has extensively been exploited utilizind non covalent interactions.[3-5] Depending upon various factors, during cocrystallization, an amalgamation of two complementary interacting materials lead to the formation of a specific product which can be any of the multicomponent organic adduct (solvate, molecular salt, eutectic or a cocrystal). The formation of either cocrystal or eutectic during cocrystallization is mutually exclusive and a win-win situation.

Pharmaceutical eutectics are the decades-old multicomponent crystalline solids having assorted utilities in routine life. They tend to form when the simplistic functional group complementarity recognition model fails to give cocrystals due to subtle structural factors. Earlier eutectics were defined only from a thermodynamics point of view, as low melting multi components which form a completely miscible single phase at a minimum coherent point on plotting temperature versus components molar ratio.[6,7] Recently Nangia et al described eutectics in terms of microstructures as "conglomerates of solid solutions"[8] and lead to the formation of the thermodynamically less stable interface due to incoherent interphase boundaries in comparison to normal coherent interaction that makes up the individual crystalline phase. Thus they possess high thermodynamic functions as well as crystalline nature leading to dual advantages of solubility and stability.

Ample reports are available in the literature describing the enhancement in dissolution rate in eutectics as compared to pure drug molecules.[8-12] The faster dissolution rate gives adequate therapeutic retention to the drug molecules sufficient for its absorption and pharmacological action. Moreover, eutectics strengthen the legal aspects linked

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with cocrystals to considerably expand their intellectual property portfolios.[11] Therefore eutectics form a viable alternative to modulate the drug molecule which suffers the low aqueous solubility across desired lines.[8] Consequently, the associated low transformation temperature, effortless purification, transparency, and availability of extensive choice of material, the organic eutectics have drawn considerable interest from various research groups.[13,14] Leflunomide (LEF) a disease-modifying drug which not only reduces the symptoms but also has the effect in dampening down the underlying disease process of rheumatoid arthritis, an autoimmune disease.[15] Due to the limited aqueous solubility of LEF, its bioavailability is affected. Enhancing solubility by using the traditional method of salt formation is not viable for this neutral drug which has a pK_a value of 10.8 at 25°C and lacks ionisable groups. Although its cyclodextrin complexes[16] to ameliorate its biopharmaceutical properties have already been reported in the literature, the associated toxicity, high cost and bulk restrict their use. In such a case, the drug can be inter-molecularly manipulated via cocrystals to optimize their physicochemical properties. Furthermore, the abundance of neutral GRAS[17]coformers for making cocrystals is much more than the number of counterions for preparing pharmaceutical salts.[18,19] For a drug molecule to undergo non-covalent derivatization, it should be predisposed with hydrogen bond donating and/or acceptor functionality. LEF which is neutral molecule possess one amide hydrogen bond donor in addition to two hydrogen bond acceptors viz. isoxazole ring nitrogen atom and carboxamide carbonyl. These functional groups give rise to various hydrogen acceptor/donor sites for an approaching counter molecule to bind and making it amenable to cocrystallization which in turn regulate the properties of a solid. With this background, our research group tried to explore the various cocrystals of this neutral, poorly soluble LEF with various GRAS status coformers. Various monocarboxylic, dicarboxylic acids as well as carboxamide (variation in position) functionality (Figure 1) were selected and screened for cocrystals using liquid assisted grinding approach. However, characterization shows that LEF resulted in eutectics instead of expected cocrystals. Hence, the present manuscript details these creening, preparation, characterization and evaluation of pharmaceutical eutectics of LEF using various techniques.

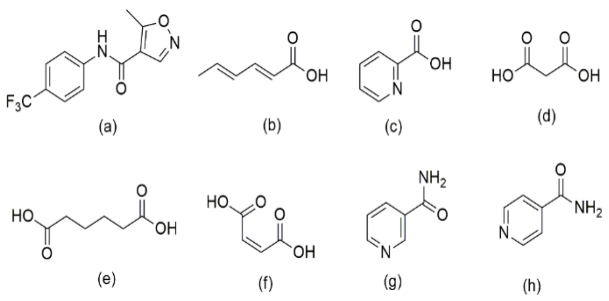

Figure 1. Molecular structures of (a) LEF (b) ADA (c) PA (d) MOA € SA (f) MA (g) NA (h) INA.

Materials and methods

Materials

LEF was obtained as a gift sample from Associated Fine Chem Co. Ahmedabad. The coformers maleic acid (MA, PubChem CID: 444266), malonic acid (MOA, PubChem CID: 867), sorbic acid (SA, PubChem CID: 643460), adipic acid (ADA, PubChem CID: 196), picolinic acid (PA, PubChem CID: 1018), nicotinamide (NA, PubChem CID: 936) isonicotinamide (INA, PubChem CID: 15074) and solvent used for preparing pharmaceutical eutectics was of AR grade and purchased from concerned suppliers. All the chemicals were used as received.

Sample preparation

To prepare eutectics LEF-MA, LEF-MO, LEF-SA, LEF-ADA, LEF-PA, LEF-NA and LEF-INA, solvent assisted (catalytic amount) solid-state grinding of LEF with maleic acid, malonic acid, sorbic acid, adipic acid, picolinic acid, nicotinamide and isonicotinamide, respectively, was done. LEF was manually ground with each coformer utilizing 1-2mL of solvent (methanol) for 30 minutes over the various mole fractions (0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.7:0.3, 0.8:0.2, 0.1:0.9). The ground products were stored in airtight containers for further analysis and evaluation.

Characterization

Differential Scanning Calorimetry (DSC)

DSC thermograms of all the samples were obtained using DSC Q20 (TA Instruments, USA) calibrated using pure indium. In sealed non hermetic aluminium pans, samples (3-5 mg) were placed and scanned at a ramp of 10°C/ min. Dry nitrogen was used as a purge gas with a flow rate of 50 mL/min. The data were analysed by TA Q series Advantage software (Universal analysis 2000).

Scanning Electron Microscopy (SEM)

Jeol JSM-6100 scanning electron microscope was used to obtain photomicrographs of LEF, coformers and their eutectics. Samples were mounted on a metal stub with adhesive tape and coated under vacuum with gold.

Powder X-ray Diffraction (PXRD)

PXRD patterns were collected on the X'Pert PRO diffractometer system (Panalytical, Netherlands) with a Cu Kα radiation (1.54 Å). The tube voltage and current were set at 45 kV and 40 mA respectively. The divergence slit and anti-scattering slit settings were set at 0.48° for the illumination on the 10 mm sample size. Each sample was packed in an aluminium sample holder and measured by a continuous scan between 5 and 50° in 2θ with a step size of 0.017°. The experimental PXRD patterns were refined using X'Pert High Score software.

Fourier Transform-Infra Red Spectroscopy (FT-IR)

Spectrum RX I FT-IR spectrometer (Perkin Elmer, UK) was employed in the KBr diffuse-reflectance mode (sample concentration 2 mg in 20 mg of KBr) for collecting the IR spectra of samples. Dry KBr (50 mg)

was finely ground in mortar and sample (1-2 mg) was subsequently added and gently mixed in order to avoid trituration of the crystals. A manual press was used to form the pellet. The spectra were measured over the range of 4000-400 cm^{-1}. Data were analyzed using Spectrum software.

In Vitro evaluation

Equilibrium solubility studies

The equilibrium solubility of LEF and prepared eutectics was determined in three different aqueous media, 0.1N hydrochloride buffer, and phosphate buffers having pH 4.5 and 6.8 simulating gastric and intestinal fluids at 37°C. The uniformity of particle size of solid samples was obtained by sieving solids through Gilson mesh sieve (no. 80) and excess amount of these sieved solids (*ca.* 50mg) were added to 25mL of different three solutions contained in a flask, preequilibrated at 37°C and the resulting slurry were shaken in a water bath shaker (MSW-275, Macroscientific Works, Delhi) at 37°C for 24 hours at 200 rpm. The samples were withdrawn after 4, 8 and 24 h and filtered through a 0.45 µm nylon filter were assayed for drug content by HPLC at 280 nm. The amount of drug dissolved for each flask was calculated using the calibration curve prepared in the respective buffers.

Intrinsic dissolution rate

The intrinsic dissolution study was performed with a rotating disk dissolution test apparatus (DS 8000, Lab India Analyticals) in three different aqueous media, 0.1 N hydrochloride buffer (simulated gastric fluid), and phosphate buffers (pH 4.8 and 6.8, simulated intestinal fluids) at 37°C and 100 rpm for 2 and 4 hours, respectively. A pellet of the sample was formed using a die and punch, compressed with a tablet press and attached to a dissolution apparatus holder and immersed in dissolution medium. 10mL of media with replacement was withdrawn at different intervals of time and after filtration through a 0.45 µm nylon filter were assayed for drug content by HPLC at 280nm.

In vivo plasma studies

The *in vivo* experiment was performed in accordance with the guidelines of the Committee for the Purpose of the Control and Supervision on Experiments on Animals (CPCSEA). The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University approval number IAEC282 dated 30/08/2012.

In order to determine blood levels of LEF in eutectics, a pharmacokinetic study was performed. The animals used in the experiment were adult male Wistar rats (weighing 180-200 g) kept under standard laboratory conditions. The animals were housed (4 rats per cage) with free access to standard laboratory diet.

The animals were divided into nine groups of six each. Group I was kept as control and treated with vehicle only while Group II was given the pure LEF (4mg kg^{-1} BW). The rest other groups received eutectics as a single dose (4 mg kg^{-1} BW) suspended in 0.5% (w/v) CMC administered by oral gavage. The dose volume for all administration was maintained at 5 mL kg^{-1}. In eutectic, the amount of LEF present is less and have been calculated to be 2.90 mg, 2.80 mg, 2.75 mg, 2.75 mg, 2.75 mg, 2.83 mg and 2.60 mg for LEF-MO, LEF-MA, LEF-PA, LEF-NA, LEF-INA, LEF-SA and LEF-ADA, respectively. Serial blood samples were collected from the retro-orbital venous plexus of the rats at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12 and 24 hours into heparinized plastic tubes. The blood samples were then centrifuged at 10000 rpm for 10 min. the plasma was separated and stored at -20°C until drug analysis carried out by the RP-HPLC method. Pharmacokinetic parameters such as C_{max}, AUC_{0-t} and relative bioavailability of eutectics were calculated by using non-compartmental analysis.

High performance liquid chromatographic analysis

The quantification of LEF in the solubility, dissolution and the in vivo plasma samples was carried out using the HPLC System of Waters Alliance (Waters Corp., Milford, MA., USA). This instrument consisted of a quaternary pump system and a photo diode array detector. The separation of solubility and dissolution samples was carried out using a SunFireC18 5-µm column (4.6 mm × 150 mm) while for plasma samples Hypersil Gold C18 5-µm column (4.6 mm × 250 mm) was used. The mobile phase comprising of water: acetonitrile (1:1, v/v) was pumped at a flow rate of 0.1 mL/min with a sample injection volume kept at 20 µL and LEF detected at 280 nm. The interpretation of the final chromatograms was done using the EMPOWER software.

Accelerated stability study

As per ICH guidelines, for one month the prepared eutectics of LEF were subjected to 40°C/75% RH in a controlled thermal humidity chamber (TH0000400G, Thermolab Scientific Equipment (P) Ltd., Thane, India) and then characterized by PXRD.

Statistical analysis

Data were expressed as mean ± SD (standard deviation). One-way analysis of variance (ANOVA) was used for each parameter using SigmaPlot (version 11.1) followed by Tukey's multiple comparison tests at $P < 0.05$ significance on Experiments.

Results

As discussed in the experimental section that seven multicomponent adducts of LEF were prepared by solid-state grinding methods. Now to establish the identity of the ground products whether they are salts, cocrystals or eutectics, they were subjected to DSC, FT-IR and PXRD analysis. It is very well established that distinct thermal, as well as spectroscopic behaviour and PXRD patterns, are observed for salts and cocrystals while the eutectic shows only a lowering in the melting endotherm in comparison to the starting components as identification characteristic.

Characterization

Thermal analysis

DSC thermograms of pure LEF, SA, PA, MA, MOA, ADA, NA and INA show sharp endothermic peaks at 169.31°C, 136.60°C, 138.16°C, 143.62°C, 138.95°C, 155.56°C, 130.65°C and 132.88°C, respectively, (Figure S1) corresponding to their melting. The liquid assisted ground product of 1:1 molar composition of LEF-NA, showed single melting endotherm at 115.22°C, which is much lower than the individual components (Figure S2). However, to determine whether this single lower endothermic peak corresponds to eutectic or cocrystal, phase diagram was constructed. For this, the various compositions of LEF and NA were prepared with different stoichiometry and subjected to DSC. In LEF-NA as the concentration of LEF decreased gradually from molar fraction 1:0 to 0.9:0.1 the two endotherms appeared at 115.22°C and 147.77°C. As the ratio further decreases from 0.8:0.2 to 0.6:0.4 the first endotherm remains nearly the same whereas the high melting shift to 145.07, 140.54 and 122.34°C, respectively. A single melting endotherm at 115.77°C appeared for a molar fraction of 0.5:0.5. Further, as the mole fraction of LEF decreased from 0.5:0.5 to 0:1 another endotherm other than invariant melting endotherm of 115.77°C appeared at 117.09, 120.54, 124.85, 125.23 and 130.65°C at mole fractions 0.4:0.6, 0.3:0.7, 0.2:0.8, 0.1:0.9 and 0:1, respectively. The increase in melting temperature might be due to the increasing concentration of unreacted NA. The binary phase diagram (Figure S3) was constructed by plotting temperature vs. Mole fraction of LEF to obtain valuable primitive information to distinguish cocrystal from eutectic.[20-24] The shape of the curve which is "W" for a cocrystal and "V" for the eutectic provides useful information whether a new solid phase is a cocrystal or eutectic. In the present study, the appearance of "V" shaped diagram is pointing towards the existence of eutectic. Similarly, phase diagrams for all the other binary mixtures (LEF-MAO, LEF-INCT, LEF-NA, LEF-SO, LEF-MA and LEP-PA) (Figure S3) were constructed. Interestingly, all the systems showed a 'V' shaped curve characteristic of a eutectic system. However, the stoichiometry varies for each system. For example, the single melting endotherm at 125°C for 0.4: 0.6-mole fraction of LEF-MA system represents exact stoichiometry. It is very clear from the binary phase diagram that variable liquids points forming "V" shaped curve represents the non-eutectic or near-eutectic compositions while the solidus i.e. the low melting invariable point represents the eutectic. Based on the solidus-liquid behaviour, the eutectic composition of each system is determined and tabulated in Table 1.

Binary phase diagram clearly shows four different regions viz., a region I, above eutectic point, the liquid phase comprising LEF and coformer, region II containing solid coformer as well as the conjugate liquid phase. Solid LEF and the conjugate liquid phase forms region III and lastly the region IV containing solid drug and coformer. Thus, from thermal analysis, all the prepared combinations are shown to result in eutectics.

Determination of thermal stability of eutectics

During the modulated temperature differential scanning, i.e., heating-cooling-heating scans (Figure 2), all the prepared eutectics showed sharp single melting endotherm at a lower temperature in the first heating cycle. Further, no thermal events were observed during the cooling and second heating cycle suggesting that neither LEF nor coformer crystallizes out from the melt of the eutectic system. Moreover, it can be inferred that none of the components of seven prepared eutectics undergoes significant molecular change during its crystallization process.

The heat of mixing (ΔH_m)

The sign and magnitude of the heat of mixing of eutectics help to comprehend the structure of the binary melt.[25] ΔH_m (heat of mixing) of eutectics was calculated by using equation 1.

$$\Delta H_m = (\Delta_f h)_{exp} - \Sigma x_i \Delta_f h_i^\circ \quad \text{Eq. (1)}$$

Where x_i and $\Delta_f h_i^\circ$ represents the mole fraction and heat of fusion for the pure component. The heat of fusion for pure components and eutectics were determined experimentally and their values are given in Table 2.

In this study, the experimental values of heat of fusion of eutectics are lower than that of the heat of fusion of sum of individual components resulting in negative heat of mixing. This indicates the formation of a cluster structure of the prepared eutectic melt.

Table 1. Melting point of components and eutectics along with the ratios of eutectics.

Compounds	Melting point (°C)	Molar Ratio	Product
LEF	169.31	-	Form II
ADA	155.56	-	-
LEF-ADA	138.1	1:1	Eutectic
SA	136.6	-	-
LEF-SA	116.09	0.4:0.6	Eutectic
PA	138.27	-	-
LEF-PA	124.96	1:1	Eutectic
MA	143.62	-	-
LEF-MA	125.97	0.4:0.6	Eutectic
MOA	138.95	-	-
LEF-MOA	124.6	0.4:0.6	Eutectic
NA	130.65	-	-
LEF-NA	115.51	1:1	Eutectic
INA	132.88	-	-
LEF-INA	125.81	1:1	Eutectic

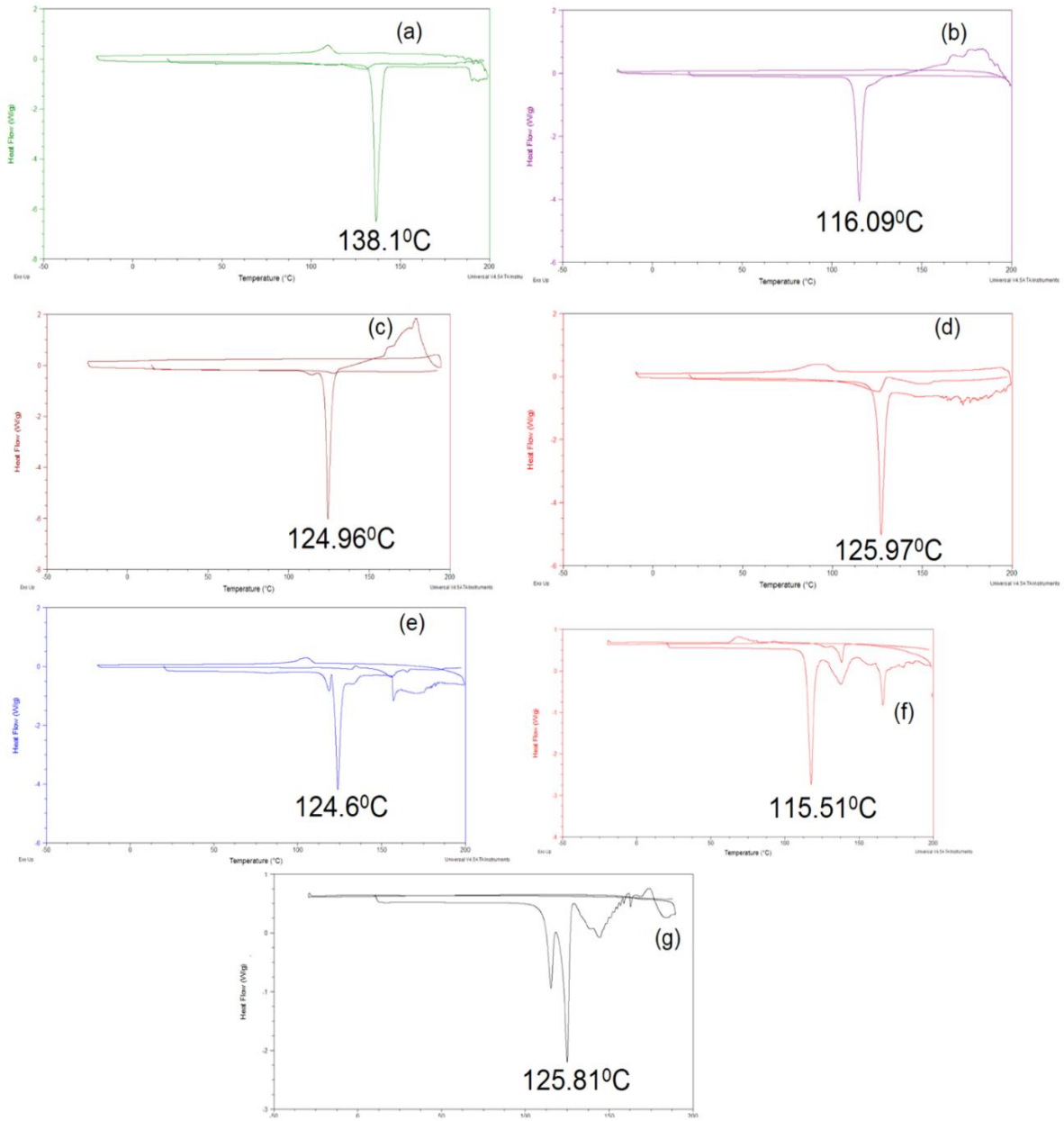

Figure 2. Modulated temperature differential scans of (a) LEF-ADA (b) LEF-PA (c) LEF-SA (d) LEF-MA (e) LEF-MOA (f) LEF-NA (g) LEF-INA, in which temperature modulation is done by (1) heating (2) cooling (3) heating.

Table 2. Values of thermodynamic parameters of eutectic systems of LEF and their individual components.

Compound	Experimental heat of fusion ($Jmol^{-1}X10^{-2}$)	Calculated heat of fusion ($\Sigma x_i \Delta_f h_i°$) ($Jmol^{-1}X10^{-2}$)	Heat of mixing ($Jmol^{-1}X10^{-2}$)
LEF	26.925	-	-
SA	119.763	-	-
LEF-SA	18.011	64.06	-46.049
PA	153.602	-	-
LEF-PA	27.942	90.264	-62.322
MA	119.583	-	-
LEF-MA	26.070	63.988	-37.918
MOA	151.932	-	-
LEF-MOA	33.212	76.928	-43.716
ADA	141.66	-	-
LEF-ADA	28.438	84.046	-55.608
NA	124.959	-	-
LEF-NA	13.899	75.942	-62.043
INA	175.074	-	-
LEF-INA	23.501	101.000	-77.499

Scanning Electron Microscopy

Scanning Electron Microscopy was used to establish the surface morphology of the pure drug, coformers and eutectics. The SEM micrographs for pure LEF, coformers and their respective eutectics at resolution 100X are given in Figure 3. In eutectics, the surface morphology and size for the two components can be seen as different. The reduction in size, as well as well dispersion of components in the eutectic mixture, was observed.

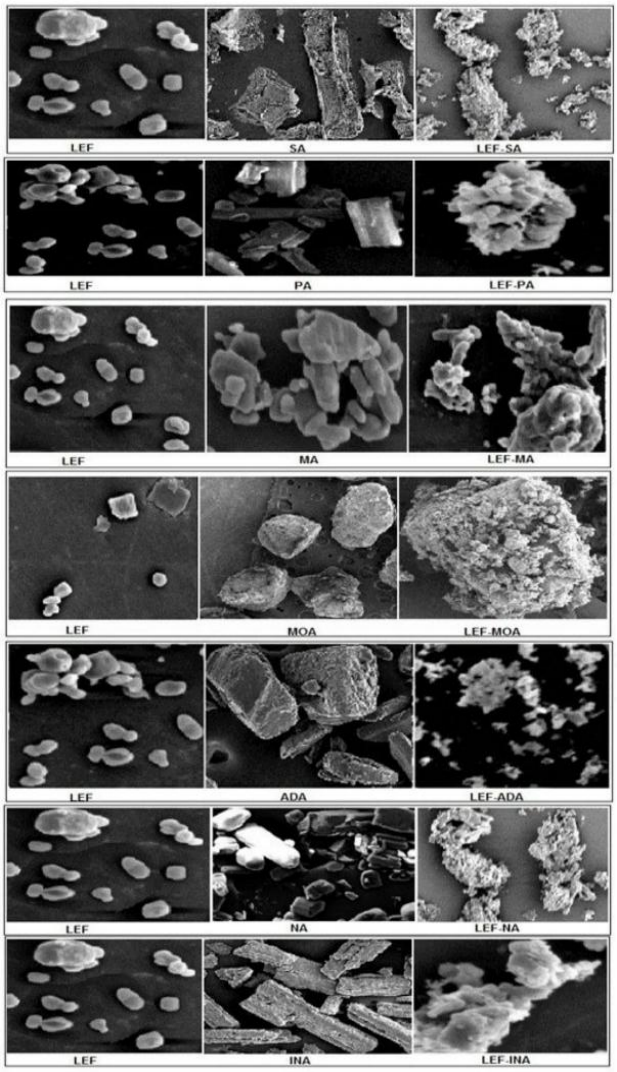

Figure 3. SEM micrographs at resolution of 100X.

FTIR and PXRD

The FTIR spectra (Figure S4) and PXRD patterns (Figure S5) of LEF, coformers and eutectics indicate that concerned eutectics contains characteristic bandsof both the drug and the coformer. Beside this no change in position of the characteristic peaks was observed. This is because, in the case of eutectics or solid solution formation, one of the two combining components includes substitutionally or interstitially in the other component. Consequently, in the crystal lattice of the resulting product, the molecular arrangement in comparison to that of individual combining components is largely unaltered. Therefore these techniques are not sensitive enough to diagnose the changes due to interaction.

In vitro and In vivo evaluation

The solubility and intrinsic dissolution rate are the mandatory parameters to be evaluated so as to estimate the developability of an API as well as to optimize its performance. Therefore the prepared eutectics were subjected to equilibrium (thermodynamic) solubility and intrinsic dissolution rate studies in simulated gastrointestinal fluids, (pH 1.2, pH 4.5 and pH 6.8), so as to evaluate any enhancement in the solubility of prepared eutectics in comparison to free drug. The enhancement in solubility (i.e. approximately 6 to 9 folds) was observed in these media up to 4 hours. However, the equilibrium solubility of LEF in all the eutectics was found to be approximately the same as pure LEF. It is because the eutectics transform into the original constituents on extended exposure to the aqueous medium.

However, not much difference was observed in the values of solubility and IDR in pH 4.5 and pH 6.8. In simulated gastric media (pH 1.2) LEF-INA and LEF-NA showed the highest comparable solubility while in simulated intestinal fluid (pH 6.8) LEF-MA and LEF-MO showed maximum enhancement (Table 3). Similarly, the IDR results also followed the same trend (Table 3). The differences in the solubility and dissolution rate of different eutectic mixtures compared to the drug are statistically significant ($P < 0.05$).

Table 3. *In vitro* equilibrium solubility and intrinsic dissolution rate of LEF and its eutectic in simulated gastrointestinal fluids (pH 1.2, pH 4.5 and pH 6.8). Values are shown in mean ± SD (n = 3).

Compounds/ Eutectic	Solubility in (µg/mL) ± SD			Intrinsic dissolution rate (mg cm^{-2} min^{-1})		
	pH 1.2	pH 4.5	pH 6.8	pH 1.2	pH 4.5	pH 6.8
LEF	23.02 ± 1.3	20.16 ± 0.5	21.08 ± 1.5	1.12 ± 0.4	0.9 ± 0.2	1.10 ± 0.1
LEF-MA	125.08 ± 0.5	186.05 ± 0.2	187.32 ± 0.9	4.25 ± 0.5	4.31 ± 0.7	4.91 ± 0.9
LEF-MOA	110.99 ± 1.8	168.94 ± 1.2	169.56 ± 0.7	3.69 ± 0.4	4.08 ± 0.5	4.14 ± 0.7
LEF-SA	84.56 ± 0.2	87.25 ± 0.9	88.37 ± 0.5	1.95 ± 0.1	1.25 ± 0.8	1.87 ± 0.5
LEF-ADA	87.92 ± 0.5	86.07 ± 0.5	86.94 ± 1.8	1.84 ± 0.8	1.93 ± 0.5	1.00 ± 0.2
LEF-PA	76.54 ± 0.3	72.89 ± 1.4	73.12 ± 1.0	1.93 ± 0.9	1.81 ± 0.02	1.87 ± 0.08
LEF-INA	162.91 ± 0.7	121.56 ± 0.5	123.19 ± 1.1	4.89 ± 0.4	3.1 ± 0.2	3.9 ± 0.5
LEF-NA	153.34 ± 0.9	115.59 ± 0.2	117.43 ± 0.5	4.31 ± 0.9	3.21 ± 0.2	3.57 ± 0.2

The differences in the solubility and dissolution rate of different eutectic mixtures values of eutectics mixture compared to the drug are statistically difference ($P < 0.05$).

No report is available in the literature regarding the eutectics of LEF to directly compare our enhancement of solubility and IDR of LEF. However, improvement in the dissolution rate is very well documented for many drug molecules such as antitubercular drug pyrazinamide, isoniazid, curcumin and hesperetin.[8-11] The plasma concentration at different time intervals of drug and eutectics was assessed by sophisticated RP-HPLC method. The results are elucidated by mean (± SD) plasma concentration-time profile after oral administration of various eutectics in Figure 4. The various pharmacokinetic parameters (C_{max} and AUC_{0-24}) were determined and tabulated in Table 4. The differences in the mean C_{max} values of eutectics mixture are statistically significant compared to the pure drug ($P < 0.05$).

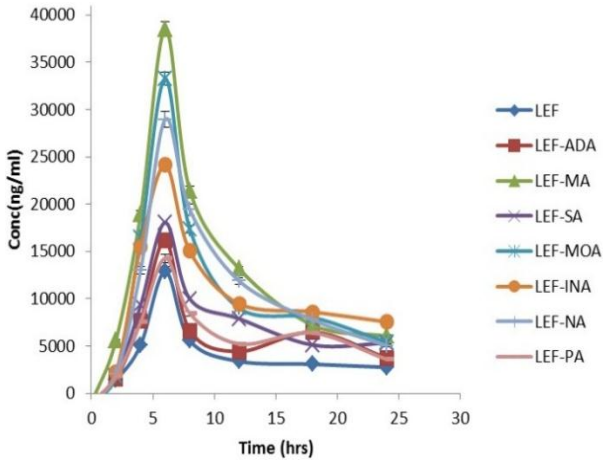

Figure 4. Mean plasma concentration- time profile of LEF and its eutectics in rats. Plasma concentration of LEF at various time points in eutectics and free drug. The comparison was carried out by one way ANOVA, and data is presented as mean mean± SD (n=3).

Table 4. Relative pharmacokinetic parameters for LEF and eutectic of LEF. Values are shown in mean ± SD (n = 3).

Compound	C_{max}(μg mL^{-1})	AUC_{0-24} (μg h^{-1} mL^{-1})	Relative bioavailability
LEF	12.088 ± 0.04	193.205 ± 0.06	-
LEF-MA	31.429 ± 0.06	490.292 ± 0.04	2.53
LEF-MOA	29.942 ± 0.01	455.118 ± 0.02	2.35
LEF-SA	18.046 ± 0.03	260.584 ± 0.04	1.35
LEF-ADA	16.162 ± 0.02	233.379 ± 0.05	1.21
LEF-PA	14.267 ± 0.01	217.478 ± 0.03	1.13
LEF-INA	27.835 ± 0.02	421.020 ± 0.04	2.18
LEF-NA	25.725 ± 0.02	394.728 ± 0.07	2.04

The differences in the mean C_{max} values of eutectics mixture compared to the drug are statistically significant ($P < 0.05$).

Accelerated Stability Study

The physical stability of prepared eutectics of LEF with selected coformers was established at accelerated conditions of 40°C/75% RH for 1 month. The PXRD patterns of the exposed samples were analysed to ascertain any effect of accelerated conditions on the physical integrity of prepared eutectics. Observation of no significant changes in the pattern of PXRD, when compared with that of the unexposed sample, suggests that the prepared LEF eutectics be stable with retained physical stability under the accelerated conditions.

Discussion

The attempt to improve the biopharmaceutical parameters of LEF by preparing its cocrystals failed as mentioned in the previous section. However, "Failures are finger posts on the road to achievement", similarly in our study unsuccessful attempt to prepare cocrystals led to more soluble and stable homogenous biphasic crystalline eutectics. The process of cocrystallization based on crystal engineering fundamentals, often considered as a supramolecular reaction where one can break the crystal structures into supramolecular synthons and ultimately leads to products taking into account both chemical (interaction hierarchy) and geometrical factors (efficient close packing).[26] Here in the present case of LEF, the appearance of the low melting endotherm for each ground binary mixture indicated the formation of a new solid phase. The appearance of characteristic peaks of individual reacting components and absence of new peaks in FTIR and PXRD pattern (Figure S4 and S5) suggested the formation of eutectics of LEF with maleic acid, malonic acid, picolinic acid, sorbic acid, adipic acid, nicotinamide and isonicotinamide. Further to confirm the presence of the eutectic system, phase diagram analysis was performed (Figure S3). From the phase diagram it is clear that as the mole fraction of each component in the various binary mixtures varied, the liquidus point also changes while the solidus remained the same. In each binary mixture of LEF with coformers, the plot of liquidus and solidus points against varying mole fractions depicts the "V" shaped graph. This shape accounts for eutectic formation. Out of all the compositions (drug: coformer) examined; only one composition represents a point (Figure S3) where the solidus and liquidus state coexists. This composition represents the true stoichiometry of eutectic (Table 2).

The formation of eutectics in the present study rather than cocrystals is due to the fact that in all the binary eutectics LEF is present in polymorphic form II.[24] In this form, the LEF molecules are essentially planar because of diminutive intramolecular contact between carboxamide oxygen and phenyl hydrogen (H) of the LEF and arranged in symmetrical layers stabilized by Π-Π interactions in an antiparallel fashion.[27] This results in acid-amide hetero synthon shape mismatch for efficient packing and heteromolecular interactions leading to the failure of cocrystal formation with maleic acid, malonic acid, adipic acid, sorbic acid and picolinic acid. Moreover, in case of binary mixtures with nicotinamide and isonicotinamide it could be presumed that an amide heterosynthon between the drug carboxamide and the coformer carboxamide may lead to the formation of supramolecular network consequently leading to the formation of cocrystals. But in the present system amide-pyridine heterosynthon which is less probable, resulted in the formation of discontinuous solid solutions leading to the generation of eutectic.

The negative value of enthalpy of mixing (ΔH_m) suggests the cluster formation which arises due to certain weak intermolecular forces resulting in the molecular association. This is the outcome of the emancipation of heat during cluster formation. The negative heat of mixing also negates the existence of quasieutectics and molecular solutions where it is more than zero and equal to zero, respectively.[28]

The solubility and intrinsic dissolution rate are two significant feature of an API especially those administered orally. Moreover, one of the factors governing the bioavailability of API is rapid and efficient absorption from the gastrointestinal membrane. This quick and systematic absorption leads to more vigorous and effective pharmacodynamic effect. All the eutectics exhibit enhancement in solubility and IDR. However, not much difference was found in the solubility and IDR at pH 4.5 and pH 6.8. It is well documented that supersaturation occurs as an outcome of size reduction of material to a very fine state of subdivision.[26] Theoretically, LEF is released from the eutectic mixture, a conglomerate of solid solutions, in a molecular state and it is logical to anticipate such supersaturation. These findings are consistent with microscopic (SEM) observations, (Figure 3) which showed a marked reduction in particle size of eutectic mixtures.

In Eutectic molecular size/shape mismatch and asymmetry in the crystal structures cause strain and disorganization of the lattice structure. The eutectic microstructure consists of domains of solid solutions held together by weak inter-phase boundaries along which atoms can diffuse and redistribute in the solid solutions.[9] Thus Imperfect atomic arrangements and poor inter-phase bonding across the domain boundaries, lead to high thermodynamic functions such as free energy, enthalpy and entropy of the eutectic phase. This result in higher solubility and bioavailability.

Moreover, the close proximity of two non isomorphous substances, as well as accommodation of differently size molecule in their respective individual lattice arrangements in eutectics, are accountable in the same manner as they are for amorphous APIs and solid dispersions.[8,30-32]

The enhancement in aqueous solubility, as well as intrinsic dissolution of LEF in eutectics, makes more of LEF available in the solution form. From this solution form, it is much easily absorbed and consequently has a direct impact on the amount entering into the systemic circulation. Moreover, this further leads to improved biopharmaceutical parameters comprising of maximum concentration (C_{max}) as well as bioavailability. The results are elucidated by concentration against time graph in Figure 4 and the pharmacokinetic parameters tabulated in Table 4. It is clear that there is an improvement in the Cmax and AUC for all the eutectics. The maximum concentration (C_{max}) achieved was nearly 3 times for LEF-MA as compared to pure LEF. The relative bioavailabilities (AUC_{0-24} of eutectic/AUC_{0-24} of LEF) were found to be 2.53, 2.35, 1.35, 1.21, 1.13, 2.18 and 2.04 for LEF-MA, LEF-MO, LEF-SA, LEF-ADA, LEF-PA, LEF-INA and LEF-NA, respectively. The higher plasma concentration of drug in eutectics at a lower dose than LEF justifies its better dissolution rate and enhanced *in vivo* absorption. The physical stability testing suggested that the prepared LEF eutectics are stable and retained physical stability under the accelerated conditions.

Conclusion

The study concludes that the failure of cocrrstallization in case of LEF leads to eutectics. These eutectics have been efficiently prepared and characterized by thermoanalytical technique. Improvement in solubility and intrinsic dissolution rate in both media (simulated gastric and intestinal fluid), as well as increased oral bioavailability of LEF in eutectics, has been observed. Therefore the ease of method of preparation as well as the wide abundance of coformers regarded as safe for human consumption to prepare eutectics allows a high level of customization of active molecule. Thus the development od API along the desired lines can open the doors for formulation development.

Conflict of interests

The authors claim that there is no conflict of interest.

Supplementary Materials

Supplementary file contains Figure S1-S5 is available on the journal's web site along with the published article.

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