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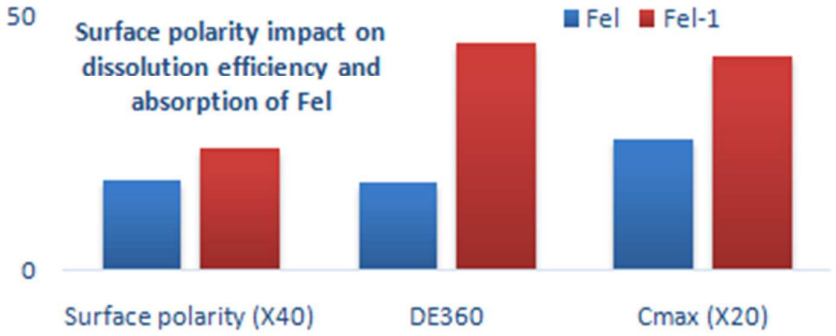
Enhanced surface polarity in Fel-1 (recrystallized from acetonitrile) resulted in enhanced dissolution efficiency and better pharmacokinetic profile



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ARTICLE

# Role of surface chemistry in crystal morphology and its associated properties

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Acetonitrile induced a remarkable enhancement of Felodipine crystal facet {11-1}. This facet was polar in nature as concluded from surface chemistry and resulted in significant improvement in dissolution rate and oral bioavailability ($p<0.05$). This conclusion was supported by surface chemistry determination by X-ray photoelectron spectroscopy and hirshfeld surface analysis.

## 1. Introduction

Many API (active pharmaceutical ingredients) show inadequate solubility and poor dissolution rate which significantly limit their oral bioavailability and consequently oral delivery.[1] Some of the approaches to improve the biopharmaceutical properties of APIs include particle size reduction, salt formation, co-solvency, cyclodextrin complexes, solid dispersions, nano-suspensions, crystallization etc.[2] The exploration of crystal forms in terms of different morphologies is a potential alternative as they exhibit different range of physicochemical properties such as flow rate, compressibility, dissolution and oral bioavailability.[3, 4]

Felodipine (fig. 1) is an oral calcium channel blocker belonging to dihydro pyridine class.[5] Chemically it is lipophilic in nature and exhibits poor solubility and poor oral bioavailability (10-15%).[6] A few drug delivery systems containing felodipine such as chitosan micro particles, micro emulsions, solid dispersions have been reported.[7-9] However, these methods present difficulties like high production costs, low stability etc. Various crystal engineering approaches like polymorphism, solvate, co-crystal synthesis has also been attempted, however the impact of its morphological characteristics on the pharmaceutical and pharmacokinetic behaviour has not been studied in detail.[9-11] This is important for Fel as it shows poor dissolution rate and oral bioavailability. The importance of crystal habit on product development is currently geared towards simulating the solid-state properties using molecular modeling.[12-14] In recent years, various eminent scientists have focused their research work on predicting the crystal habit of a particular compound under a given set of conditions.[15-17] Ulrich et al., developed build in and surface docking approach to predict the morphological modification of organic crystals induced by the additives.[18] Duan et al., used surface docking approach to predict effect of solvent on HMX molecule.[19, 20] Femi-Oyewo and Spring have reported crystal growth inhibitory action of HPMC on paracetamol crystals.[21] Interaction of polymeric additives with crystal facets is heterogeneous because of the anisotropic nature of the majority of molecular crystals.[22-24] Rasenack and Muller., reported that dissolution rate can vary with crystal habit.[25]Chen et al., also concluded that modified crystal habit can be exploited to increase the dissolution rate of poorly-water soluble drugs.[26]

Molecular simulation could significantly trim down the time and effort spent in experimentations, and connect bulk processing directly with product development.[22, 27] Hence, a combined strategy can be useful in obtaining crystals of desired architecture.[28, 29]The goal of this work was to investigate systematically how crystal morphology can influence biopharmaceutical properties of Fel. The novelty of this work lies in molecular dynamics studies of crystal habit modification of Fel and correlation of its facet properties (surface chemistry) to the crystal growth and final morphology in a specific medium. The outcome of facet specific surface chemistry was in turn correlated to the dissolution rate and oral bioavailability, which makes this study more advanced. The surface chemistry of the individual crystal facets was determined by morphology growth model, which was used to predict the molecular orientation at the surface of crystals. Surface chemistry was also interpreted by hirshfeld surface analysis to gain insight into crystal growth. Based on crystal surface chemistry; the mechanism for the alteration in morphology was suggested. The changes in dissolution rate and oral bioavailability was correlated to three parameters; aspect ratio, surface (S)/volume (V) ratio and surface area of morphologically important polar facets. Two crystal habits of Fel were obtained by re-crystallization. An attempt was made to correlate experimental and simulated crystal habits. Various solid state characterization tools were utilized to examine any solid form change. Modified crystals were investigated for their dissolution rate and the crystals with improved dissolution rate

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were further investigated for oral bioavailability. XPS values were used to establish differential surface chemistry.

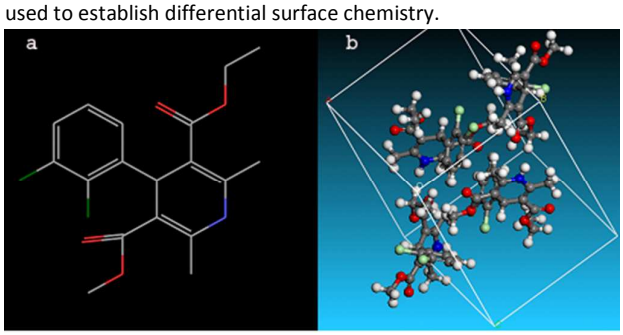


Fig. 1. a. Molecular structure and b. crystal lattice of Fel

## 2. Experimental section

Fel was received as a gift sample from Aurbindo Pharma (Hyderabad, India). Lacidipine was received as gift sample from Dr. Reddy's Laboratories, India. Methanol and acetonitrile (HPLC grade) was purchased from Merck, India. All other chemicals used were of analytical grade. In-house ultra-pure water from Millipore® was used in all experiments. Amber colored glass wares were used for all experiments and storage. All methods are provided as supplementary data.

## 3. Results and discussions

### 3.1 Crystallization experiments and computational simulation

Vacuum morphology was generated by BFDH model (Supplementary fig 1a), which gave 5 important facets along with their planes (hkl), centre to plane distance ($d_{hkl}$) and % surface area. The crystal facets consisted of (1 0 0), (0 1 1), (1 1 0), (1 1 -1) and (1 0 -2), of which (0 1 1) with 37.48%, and (1 0 0) plane with 26.49% of the total facet area were the most important facets (Supplementary table 1). According to morphology growth model, the crystal facets consisted of (1 0 0), (0 1 1), (1 1 0), and (1 1 -1) planes, of which (1 0 0) facet with 32.95% of total facet area, and (0 1 1) facet with 43.96% of total facet area were the most important facets (Supplementary table 2). Similarly, from the equilibrium morphology model, the morphologically important crystal faces were (1 0 0), (0 1 1), (1 1 -1), and (1 0 -2), in which (1 0 0) with 17.69% of total facet area, and (0 1 1) with 28.95% of total facet area were the most important facets (Supplementary table 1). From the vacuum morphology models it was concluded that facets (1 0 0) and (0 1 1) were the most important facets that played a dominant role in deciding the morphology of the simulated habits. The calculated aspect ratio by BFDH morphology, growth morphology and equilibrium morphology models were 1.81, 2.15 and 1.44 respectively(Supplementary fig 1). However, the predicted habits from these three models were different from the experimental habit, which was attributed to the absence of actual experimental conditions that were provided during crystallization experiments. Hence, there was a need to incorporate solvent molecules to predict habits that would be close to experimental habits.

From the equilibrium solubility studies, Fel showed good solubility in acetone, ethanol, methanol and acetonitrile, whereas it was found to be poorly soluble in hexane and IPA (Supplementary table 2). Habits were successfully modified with acetonitrile (Fel-1). Rhombohedral shaped crystal habit with an aspect ratio 1.15 ± 0.14 was obtained experimentally for Fel-1 (Fig 2aand supplementary

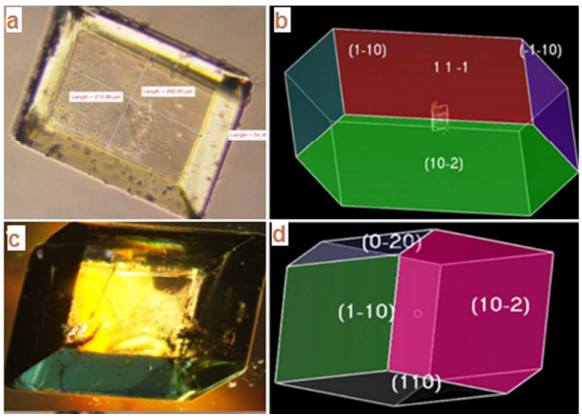


Fig. 2. a. Experimental morphology of Fel-1, b. Simulated morphology of Fel-1, c. Experimental morphology of Fel-2, d. Simulated morphology of Fel-2

fig. 2a), while a rhombohedral habit with an aspect ratio 1.27 was also simulated by the computational technique when provided with similar crystallization environment (Fig 2b). However, irregular sized and non-reproducible crystals (crystals with aspect ratio of standard deviation >0.5) were obtained with other good solvents (Supplementary table 2). The 1.15 aspect ratio obtained with acetonitrile was superior when compared to other solvent systems but not ideal (=1), hence attempts were made to re-crystallize using anti-solvents.

The solvent: anti-solvent combinations that gave crystals with least aspect ratios were selected for further studies (Supplementary table 2), since crystals of higher aspect ratio are generally not preferred for pharmaceutical use.[30] Plate crystal habit with an aspect ratio 1.37 ± 0.20 was observed for Fel re-crystallized with ethanol as solvent and hexane as anti-solvent (Fel-2) (Fig 2c), while the simulated habit was also almost plate shape with an aspect ratio of 1.52 (Fig 2d). The shape of Fel-1 and Fel-2 crystals predicted by the modified attachment energy model is shown in fig 2b and fig 2d, which indicated good agreement with the experimentally grown crystal morphologies (Fig 2a, 2c) with statistically no significant variation between the aspect ratio of experimental and simulated habits ($p > 0.05$). However, some of the faces are not identical (fig 2a and fig 2b). The morphologically important facets for Fel-1 were (1 1 0) and (1 1 -1) and for Fel-2; (1 1 0) and (1 0 -2) (Supplementary table 1). The results led to a conclusion that the solvents chosen as a crystallization medium had a major role in influencing the final crystal shape especially in terms of its aspect ratio. Although the aspect ratio obtained using solvent anti-solvent system (Fel-2) was significantly larger than that obtained with a single solvent system (Fel-1), Fel-2 was selected for further studies to support comparison studies when determining the effect of various crystal surface parameters on dissolution.Uniformity and reproducibility in size and morphology was also checked for these selected crystal systems.Attempts were made to re-crystallize Fel in presence of additives like HPMC and polysorbate 80 to further improve the morphology. However, uniform and reproducible crystals were not obtained when additives were used during re-crystallization.

The images of experimentally re-crystallized Fel (Fig 2a, 2c) clearly showed crystal morphology changes as a function of crystallization

medium. The orientation of molecules on the exterior of each facet is diverse due to its structural dissimilarity. This dissimilar relative abundance of functional groups on the facets of crystal habits (anisotropy) confers different surface properties. It was presumed that solvent molecules might show differential adsorption onto a crystal face and influence the crystal growth in terms of its dimensions.[31] In practice, the polarity of the solvent/anti-solvent used as crystallization medium, specific interactions among solvent molecules and crystal facets play significant roles in controlling facet-specific growth rates and the final crystal product.[22]

It has been observed that polar moieties are preferentially adsorbed by the polar facets and non-polar moieties by the non-polar facets, which determine the crystal morphology. Hence, examination of relative presence of functional groups on facets of simulated Fel crystal habit (Supplementary fig 3), may allow correlation of crystal surface property with properties of crystal growth medium. It was clearly visible from the facet specific surface area values of Fel-1 (Supplementary table 1) that acetonitrile induced an enhancement of growth on facet (1 1 -1) (~40%). The crystal facet (1 1 -1) due to the abundance of polar groups (1 methyl, 2 carbonyl, one chloride and 1 aromatic ring) dominated in a polar environment like acetonitrile which has high dielectric constant (37.5)[32]. Whereas, combination of methanol and hexane a lower dielectric constant solvent system (~24.5due to addition of hexane), induced the growth of non-polar facet (1 1 0) in Fel-2 (~58% of total facet area) (Supplementary table 1). This surface slice of (1 1 0) dominated in relatively non-polar environment due to the abundance of non-polar groups (Supplementary fig. 3, supplementary table 3). It was presumed that the contribution of the surface area of (1 0 -2) facet may not be significant as it shows nearly equal presence of polar and non-polar functional groups.

An alternative way to study the crystal growth pattern is by comparing the intermolecular interactions using hirshfeld surface analysis.[33] The hirshfeld surface defines each independent molecule's environment within a crystal.[34]In afirst-of-its-kind study, hirshfeld surface parameters were correlated with crystal growth pattern and final morphology and used to identify and characterize various aspects of different crystal environments. The sum of molecular interactions is depicted in the fingerprint plots in supplementary fig. 4. This two-dimensional (2D) plot helps in visualizing the three-dimensional (3D) distance information between the defined hirshfeld surface and atoms external ($d_e$) and internal ($d_i$) to that surface.

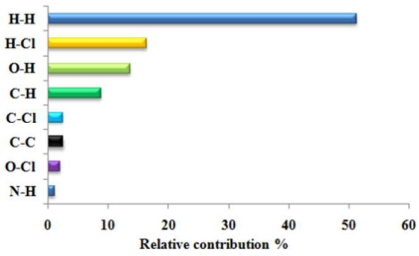


Fig. 3. Relative contributions to the hirshfeld surface

The largest percentage of contacts are found between H···H atoms (51.3) which is presumed not to affect the crystallization significantly (Fig 3). Most of the difference is anticipated to come from the O···H (13.7%) and H··Cl (16.4) contacts, which are polar in nature as compared to non-polar C··H (8.9%). It can be assumed that polar acetonitrile molecules are preferentially adsorbed by the polar facets (1 1 -1) and became dominant in Fel-1, while in Fel-2, non-polar moieties are absorbed by the non-polar facet (1 1 0) that determine its morphological outcome.

**3.2 Solid state characterization of modified crystals**

FT-IR spectra, DSC thermographs, TGA and PXRD pattern of (supplementary figure 5-8) indicated that all the crystal samples were similar without any differences in their internal structure and conformation (absence of polymorphism). XPS survey spectra showed the presence of carbon (C), oxygen (O), chloride (Cl), and nitrogen (N) on the surface of plain Fel as well as on the selected habits(Fig. 4). The chemical shift and peak shape were similar between these samples, indicated no qualitative differences in Fel crystal habits and plain drug. However, the relative abundance of surface elements showed significant differences. The surface of Fel-1 sample exhibited a relatively lower concentration of C and higher concentration of O, Cl, and N than Fel-2. The surface polarity, expressed as (O + Cl + N)/(C), was 0.6 and 0.42 for Fel-1 and Fel-2, respectively. A similar pattern of higher surface polarity for Fel-1 was also observed from their facet specific surface chemistry.

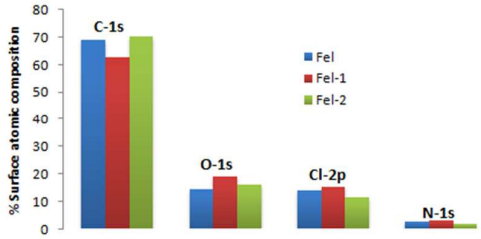


Fig. 4. Surface Elemental Composition of Fel Crystal Habits

**3.3 Comparative dissolution rate**

Crystal habits and their closely related properties such as aspect ratio, surface anisotropy and the effective surface area exposed to dissolution medium are known to control the dissolution rate of crystals.[22] Crystal habit modification resulted in significant difference in dissolution rate when particle size of similar distribution were studied (Supplementary table 4). The order of dissolution rate was Fel-1 > Fel-2 > Fel (Fig 5) when 0.1% SLS-water was used as dissolution medium. The same pattern was observed when dissolution efficiency at various time points ($DE_{60}$, $DE_{180}$ and $DE_{360}$) was compared (Supplementary table 5). Fel-1 showed significant improvement in dissolution rate when compared to Fel. Fel-2 also showed an improvement in dissolution rate, however, the extent of dissolution rate was comparatively less when compared to extent of improvement in dissolution rate exhibited by Fel-1. This level of improvement is significantly relevant to biopharmaceutical performance of drugs with poor solubility like Fel. The difference in dissolution profile can be ascribed to changes in the relative abundance of functional groups on the crystal facets in Fel-1 and Fel-2. The reason for improved dissolution rate of Fel-1 was the polar nature of its most important facet (1 1 -1). This enhancement in polar surface area was also coupled with their enhanced S/V ratio, which was 1.29 for Fel-1 and 1.20 for Fel-2. The enhanced S/V ratio of Fel-1 is probably due to anisotropy and lower aspect ratio that could have resulted in enhanced dissolution rate. These

observations are also in line with the XPS data (fig. 4) that indicated a higher presence of O, N, and Cl on the surface of Fel-2 when compared to Fel-1. Thus, the abundance of polar facets in Fel-1 could be attributed to its higher dissolution rate.This enhancement in dissolution rate for Fel is in line as with solid dispersions[7], nanoemulsion[8] and solvates[9].

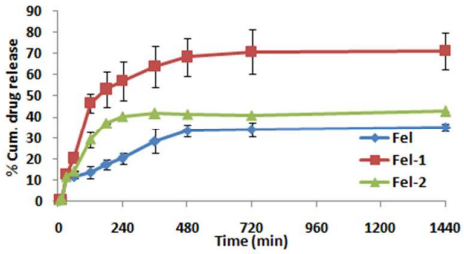


Fig. 5. Dissolution rate of selected Fel crystals (n=6)

### 3.4 In-vivo study

Significant enhancement ($p < 0.05$) in dissolution rate was observed with Fel-1 ($DE_{360} = 44.9$), when compared with Fel ($DE_{360} = 17.2$) and Fel-2 ($DE_{360} = 30.7$), hence Fel-1 was selected for oral bioavailability study and compared with Fel. Fig 6 shows the mean plasma concentration–time profiles. The enhanced *in vitro* dissolution rate of Fel-1 was translated into a significant improvement in oral bioavailability. The $C_{max}$ of Fel was 1.28 ± 0.56 µg/ml, whereas Fel-1 exhibited a higher $C_{max}$ value of 2.11 ± 0.5 µg/ml. Thus, a significant ($p < 0.05$) 1.63 fold increase in the peak plasma concentration ($C_{max}$) was observed in Fel-1 when compared to Fel. This enhancement in $C_{max}$ indicates the enhanced absorption rate of Fel-1. The AUC $_{0–12h}$ of Fel-1 crystals exhibited a significant ~1.23 times increase in the oral bioavailability, when compared to that of Fel suspension ($p < 0.05$) indicative of increased extent of absorption. The higher *in vitro* dissolution rate of Fel-1 thus provides an explanation for enhancement in both rate and extent of oral bioavailability and is in line with the trend observed in surface chemistry study wherein the proportion of polar facet increased in Fel-1.

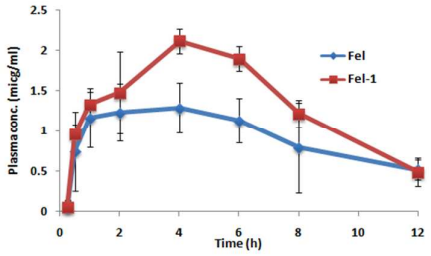


Fig.6. Plasma concentration-time profile of plain Fel and Fel re-crystallized from acetonitrile

## 4. Conclusions

The experimental morphology observed by microscopy was successfully predicted by the Materials Studio simulation program using modified attachment energy model with COMPASS force field. Interpretations from FT-IR, DSC, TGA and PXRD results led to the conclusion that no polymorphs or solvates were formed and only habit modifications occurred during re-crystallization. Fel re-crystallized with acetonitrile (Fel-1) resulted in significant enhancement of dissolution rate. This improved dissolution performance of Fel-1 translated into an *in vivo* oral bioavailability enhancement. XPS and hirshfeld surface analysis were successfully used for analyzing habit modification and their impact on pharmaceutical and biopharmaceutical performances. The study has demonstrated the potential of using molecular dynamics to provide an improved understanding of the differences and changes in crystals exhibiting dissimilar habits. This kind of improvement is relevant to biopharmaceutical performance of drugs with solubility and bioavailability problems.

## Acknowledgements

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### Abbreviations

Fel, felodipine; DSC, differential scanning calorimetry; TGA, thermogravimetric analysis; p-XRD, powder X-ray diffraction

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