CO-CRYSTAL SCREENING: FROM SMART DESIGN TO EFFECTIVE EXPERIMENTATION

Why co-crystals?

Solid form selection and development is one of the key aspects of pharmaceutical research, as over 90% of all APIs on the market are approved as solid formulations. These require a thorough understanding of the solid form landscape of the API and of the crystallization process to be reliably controlled towards the desired solid form. By choosing the appropriate polymorph, salt or co-crystal of the given API, the formulation chemist can tune the physico-chemical properties of the compound, and in turn it's therapeutic profile.

Whether a polymorph, a salt or a co-crystal, the solid form selection and development process is always based on two converging aspects of research. The first is a profound knowledge of the underlying chemistry involved, which is expressed in the Design of Experiments. The second are the experimental capabilities and methods required to execute the Design of Experiments in a reliable, accurate and reproducible manner. Over the past 20 years solid state research has advanced greatly in both directions: predictive theoretical methods coupled with better experimental equipment are now readily available to scientists.

Co-crystals can improve:

- Solubility
- Dissolution profile
- Bioavailability
- Stability
- Taste masking
- Melting point modification

Co-crystal screening state-of-the-art

Co-crystals have received significant interest as they are sometimes the only feasible way of overcoming the solubility and bioavailability issues of certain APIs which cannot be turned into salts, nor have any suitable polymorphs. However, co-crystal formation is generally complex, as the steric and chemical properties of the API and co-former have to match at the molecular and crystalline level. As such, many of the recent advancements in pharmaceutical solid-state research have focused on first predicting co-crystal formation and then reliably obtaining the desired solid forms.

The case study of Febuxostat and its co-crystal screening with p-toluenesulfonic acid

The current case study details the use of two cutting-edge recent developments in the field of co-crystal screening. It covers the full process of how a febuxostat p-toluenesulfonic acid co-crystal was first predicted using a cutting-edge machine learning algorithm, and then subsequently obtained experimentally using the ***Crystal16*** device, which is ideally suited for this type of co-crystal screening.

Febuxostat is used for the prevention of gout by decreasing the amount of uric acid the body produces. It was chosen as the test API due to its very rich, but not fully explored solid form landscape, including several known polymorphs, solvates and co-crystals.[1] This makes screening for new solid forms even more challenging, as the most experimentally accessible hits have already been discovered.

Using an AI graph algorithm for co-crystal prediction

Co-crystal prediction has come a long way since when the chemist's intuition was the only tool available for selecting an adequate co-former. Over the years, several computational methods have been developed for ascertaining and ranking the probability of co-crystal formation of API-co-former pairs. Some of the more accurate prediction techniques are based on experimental crystallographic data, usually from the Cambridge Structural Database (CSD) which curates the information of over 1 million crystal structures determined at Å resolution.

Figure 2. Febuxostat and p-toluene sulfonic acid structures

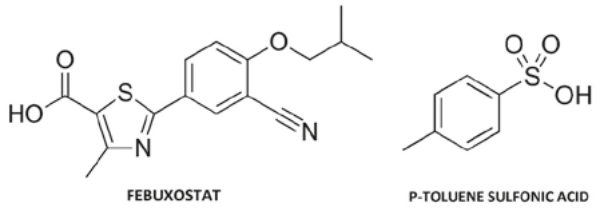
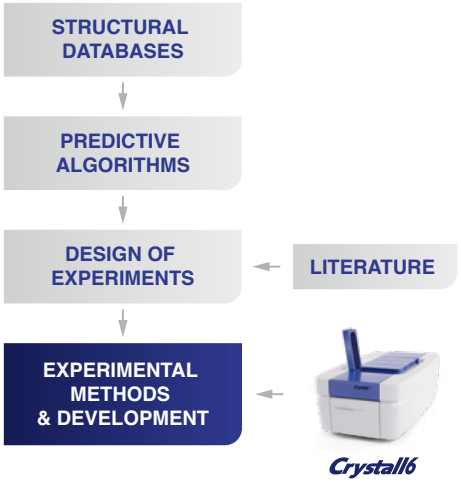


Figure 1. Co-crystal prediction and experimental development diagram

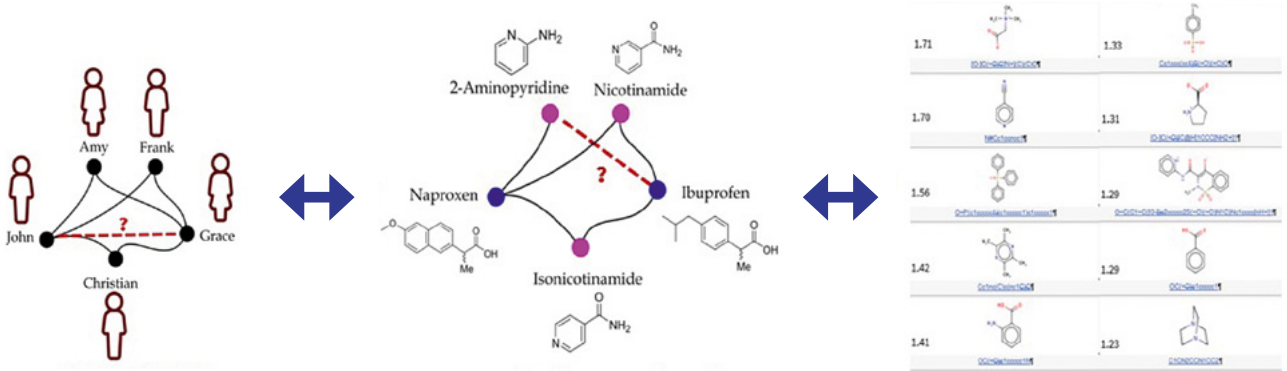


A new approach for co-crystal prediction was recently reported and is based on a machine learning link prediction algorithm. On a highly simplistic level, this AI method works as follows:

- all co-crystal structures from the CSD are collected
- the relation between APIs and coformers are mapped as an interconnected network (social network analogy)
- the resulting correlation network is analysed for missing connections between nodes, more specifically between the API and coformers for similar molecules

By analysing the graph connections between different co-crystal pairs of molecules, it is possible to predict and rank the likelihood of new links between the target API and new potential co-formers.[2] Applying this method to febuxostat confirmed existing co-crystals not used in the training set and also highlighted new potential ones. From this new hit list, p-toluenesulfonic acid was chosen for experimental development, as it is a widely used pharmaceutical coformer and counterion, and, moreover, it was overlooked by previous solid form development studies on febuxostat.

Figure 3. Schematic representation of the link prediction algorithm and ranking of some potential co-formers for febuxostat co-crystals



Social network **Coformer network** **Ranked list of hits**

Co-crystal experimental screening

No matter how accurate the predictive method is, there is still the need to experimentally confirm the findings. Experiment co-crystal screening implies testing several crystallization methods, API and coformer concentrations, in various solvents and thermodynamic conditions. Computational methods can point you towards a likely coformer, and therefore significantly reduce the number of experiments required for a successful hit. A wide range of solvents can further complicate things, through the formation of solvates. For this reason, 4 solvents were chosen for this study, due to their low likelihood of forming solvates and for their good solvation ability towards both API and coformer.

Having reduced the complexity of the screening as much as possible, there is still the need to test several crystallization modes, as well as concentrations. The ***Crystal16*** device is ideally suited for such work and is also a well-known research tool used for co-crystal screening.[4]

Method A: Cooling Crystallization

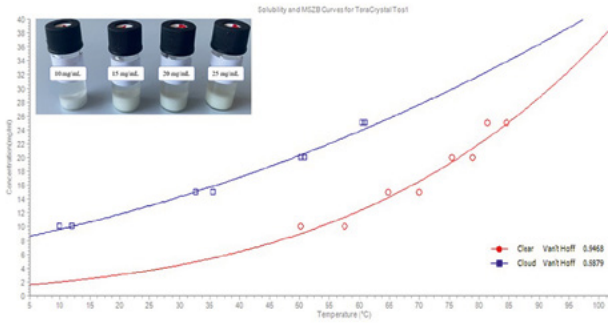
This screening approach is focused on finding suitable solvents for congruent dissolution and co-crystallization of the API and the selected conformer, in a 1:1 molar ratio. Therefore, for each selected coformer, one can envisage an experimental design involving:

- 1 ***Crystal16*** run per conformer
- 4 API concentrations in a 1:1 molar ratio with the coformer
- 4 different solvents

This set-up was applied in the case of febuxostat in combination with p-toluenesulfonic acid and four solvents: 2-ethoxyethanol, THF, EtOH and Acetonitrile. Nevertheless, in a typical screening program, at least 10 co-formers are tested, adding up to > 160 experiments.

We present below the results obtained for 4 samples containing 1:1 febuxostat:p-toluenesulfonic acid with API concentrations of 10, 15, 20 and 25 mg/mL, respectively, in 2-ethoxyethanol using to two temperature cycles between 5-110 °C. This method has

Figure 5. Solubility curve of febuxostat p-toluenesulfonic acid co-crystal in 2-ethoxyethanol



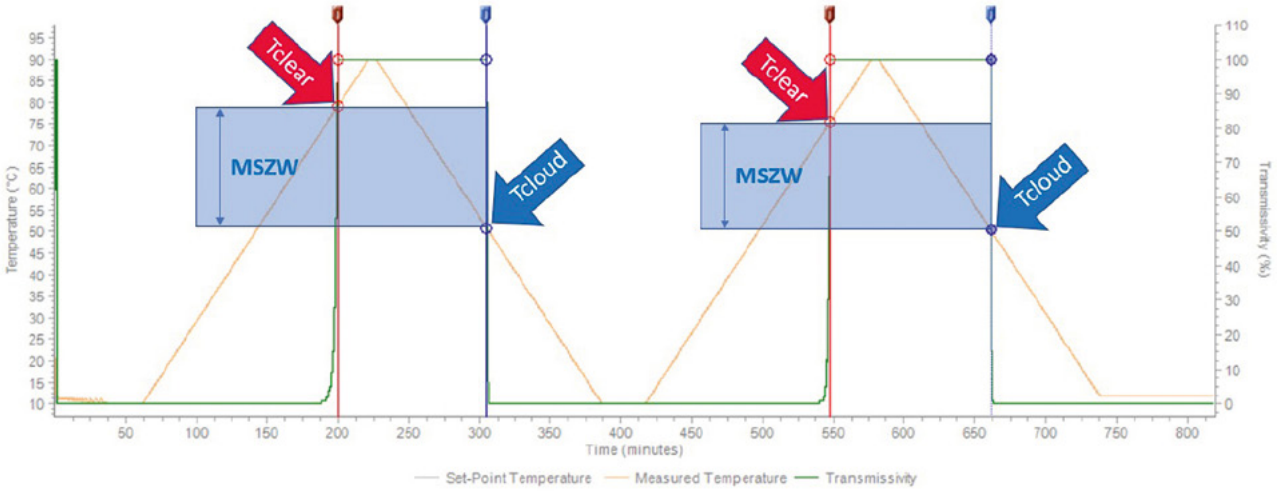
several advantages, among which is, if and all 4 experiments result in the novel co-crystal **Tos1**, a solubility curve can also be determined for the co-crystal:solvent system. This solubility information is crucial for further scale-up development.

Method B: Slurry Crystallization

Slurry co-crystallization might be preferred at the industrial scale compared to cooling crystallization, as it generally allows for more material to be obtained for a given amount of solvent. This crystallization method can also be readily tested using the ***Crystal16*** at the millilitre scale, using a similar setup as in cooling co-crystallization for each co-former: 4 concentrations, 4 solvents in one C16 run.

In the case of the 1:1 febuxostat:p-toluenesulfonic acid system, based on the solubility information obtained using Method A, 4 slurry samples containing 1:1 API:co-former with API concentrations of 90. 120, 240 and 360 mg/mL, respectively, were prepared in 2-ethoxyethanol and subjected to the same temperature program as for Method A, two heat-cool cycles between 5 and 110 °C. As in the previous case, all 4 experiments resulted in the same pure crystalline form, febuxostat tosylate co-crystal **Tos1**. Working at these larger concentrations produced sufficient crystalline material for both a full material characterization (XRPD, DSC/DTA, FT-IR etc.), as well as the determination of the aqueous solubility of **Tos1**.

Figure 6. MSZW observed in the first and second temperature cycles (20mg/mL API concentration)



Conclusions

The current case study showed that an effective co-crystal screening protocol can be defined using the ***Crystal16*** instrument. The in-situ light transmission measurement during the co-crystal screen offers an important advantage for the scale-up and process development design, by allowing the determination of the solubility curve and MSZW for the co-crystal:solvent system.

Using the ***Crystal16***, within 1 day you can test one conformer in 4 solvents simultaneously, with 4 concentrations in each solvent and maximize co-crystal formation by fully exploring the concentration range. In one day, you can reliably test co-crystal formation for a high ranked co-former and get information on its crystallization conditions for further scale-up and process development.

Co-crystal screening with Crystal16:

- 4 blocks x 4 concentrations each = 16 individual simultaneous experiments
- 1 coformer in 2-4 solvents **successfully screened per day**

A general co-crystall screening protocol can be proposed based on the Tos1 work: one ***Crystal16*** run per co-former, each with 4 concentrations, 4 solvents and equimolar ratio between API and co-former is a good approach for reliably screening and obtaining co-crystals. Furthermore, it is possible to scale up this procedure to a co-crystal screening with as many solvents and coformers as your project requires.

Acknowledgements

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Figure 7a. A general experimental design for screening 1:1 API:conformer compositions using the Crystal16

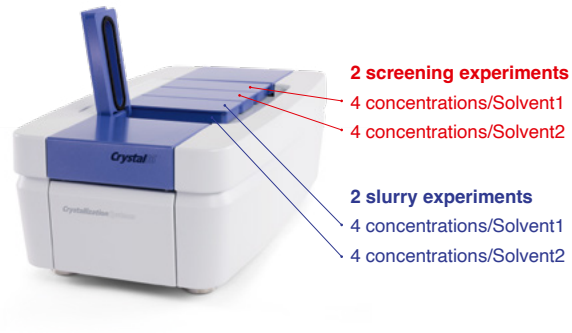
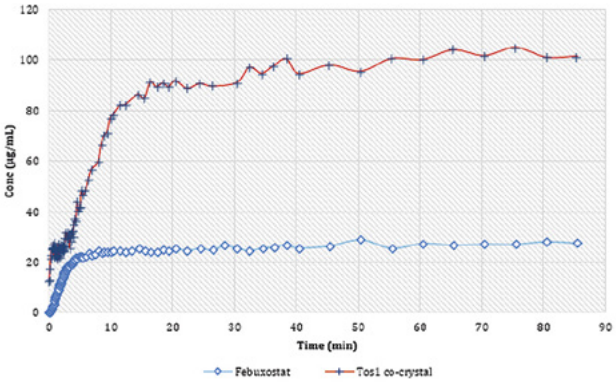


Figure 7b. Improved solubility of the new Tos1 co-crystal, the main goal of this study



*The improved dissolution curves of the **Tos1** co-crystal and the starting material (FEB) in water.*

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