User Perspectives on the Application of eXalt™ Technology to Pharmaceutical Small Molecule Crystallisation - Review Paper

Rob Darrington, Genevac Ltd, Ipswich, UK.

Introduction

eXalt technology enables evaporative crystallisation studies to be performed. In particular, eXalt allows for multiple small molecule actives to be crystallised from multiple different solvents all at the same time, at the same rate, and under the same conditions. This provides a high degree of control to the scientist which has hitherto not been available during evaporative crystallisation studies.

During the summer of 2013 researchers at Novartis Pharmaceuticals in Horsham, UK, carried out a lengthy evaluation of eXalt technology to investigate how it may be applied to small molecule crystallisation processes in pharmaceutical chemistry research and development. A presentation with an account of their work is available via www.Genevac.com/eXalt - this paper is a review of that presentation. Please note, the views in this paper are the views of Genevac and not an endorsement by Novartis Pharmaceuticals.

Equipment & Methodology

eXalt comprises a special sample holder for 4ml (1 dram) vials – Figure 1. To control the rate of evaporation for each solvent a 'tower' with baffles is constructed and placed in the holder. This seals to the top of the vial and slows the rate of evaporation. The baffles have a hole in the centre of varying diameter, ranging from 0.5mm upwards. The selection of baffles is based on a reference table and depends on the volatility of the solvent as well as the duration required for the process. Therefore, a very volatile solvent will use a number of baffles with a small diameter hole, and a less volatile solvent will use a single large diameter baffle, or perhaps none at all. A 3ml solution containing active compound, normally 5-10mg, is placed in the vial. The evaporation rate of some solvents is naturally so slow that a volume less than 3ml has to be used.

Figure 1: eXalt holder

L to R: eXalt baffles and tower assembly, loading vials and towers to holders, completed holder assembly

The assembled holders are placed into a Genevac HT-4X evaporator and a method for the desired evaporation time is selected. The action of the spinning rotor in the HT-4X seals the 'tower' to the vial. The current methods allow selection of a time between 6 hours and 96 hours in 6 hour increments, although any time may be programmed. The evaporator the cycles the pressure for 3 minutes at atmospheric pressure and 3 minutes at atmosphere

minus100mbar for the duration of the process, and maintains a steady temperature. These conditions coupled with the 'towers' create slow steady evaporation of all solvents loaded. eXalt is non-destructive, so even a sample that fails to crystallise may be recovered and used for other studies.

Initial Studies

Initial studies focussed on use of commercially available compounds and were used to explore the potential and limitations of the technology.

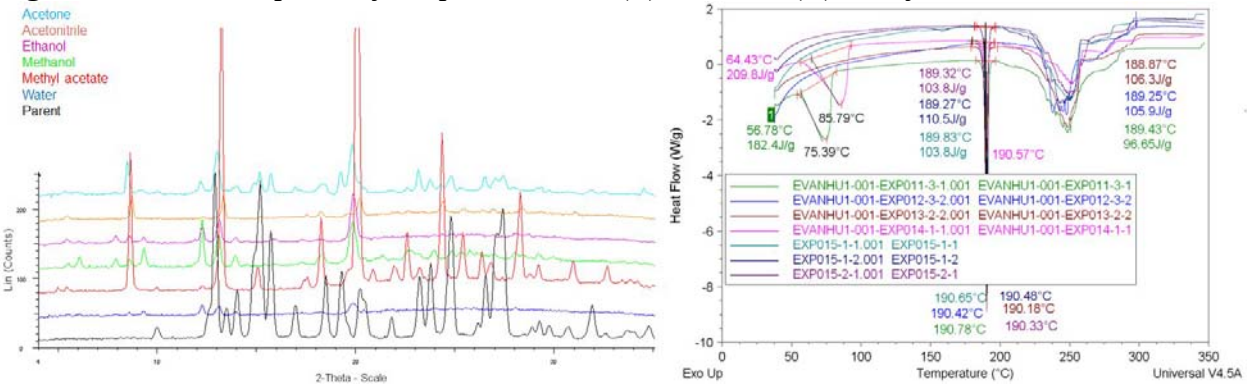
The pre-programmed methods need some further refinement with regards to being able to accurately predict the end time, the condenser auto-defrost & draining was found to be variable in duration. The methods were very easy to use. Further work is required to improve the look up table supplied, and to better refine a method for determining settings to be used for solvents not listed. The towers can be a little fiddly to produce, but must be able to be disassembled for cleaning purposes. When loading, a plan of holder and top rack are essential!

Ibuprofen, Caffeine and Carbamazepine were recrystallized from a range of solvents over 24 hours and also over 66 hours to study the potential to form different polymorphs:

- No solvent cross contamination between vials was seen, e.g. solvent A being found in a crystal formed from solvent B, where solvent A was also screened at the same time.
- The evaporation rate of the solvent did not seem to be affected by the presence of the active. The active can affect the rate during *conventional* concentration to dryness.
- Ibuprofen and Caffeine each evaporated from acetone, acetonitrile, ethanol, ethyl acetate and tetrahydrofuran showed the same polymorph as the starting material in all cases.
- Carbamazepine was evaporated from acetone (Photo A), acetonitrile, ethanol, methanol, methyl acetate and water. Analysis via x-ray powder diffraction (XRPD - Figure 2) indicated formation of different polymorphs but these were not confirmed by differential scanning calorimetry (DSC) analysis of melting point. Either no true polymorphs were formed, or, due to a lag of several weeks between XRPD and DSC the crystals had all converted to a single form.

Photo A: Carbamazepine crystallised from Acetone

Figure 2: Carbamazepan Polymorphism XRPD (L) and DSC (R) Analysis



A study was started to evaluate the possibility of using eXalt as a semi-automated screening technology for co-crystals which could be conducted early during development, rather than as a place of last resort. Carbamazepine was combined 1:1 with various co-formers and crystallised from different solvents, based on published precedent or in-house sources. Some nice crystals were created (Photo B), and the analytical work initiated, however other projects took over and this work remains incomplete. There is potential here which would ideally be investigated and developed further.

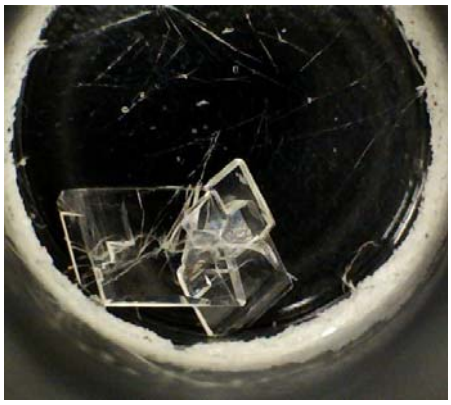
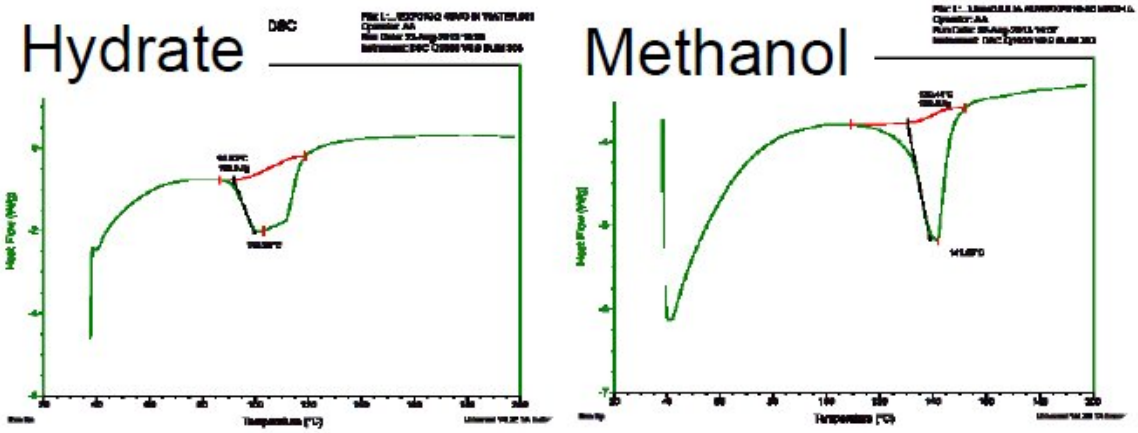


Photo B: Adipic Acid & Carbamazepine co-crystallised from ethanol

Work with Ongoing Pharmaceutical Projects

Working with an ongoing project, eXalt was used to see if it was possible to displace a hydrate from a compound which had a propensity to crystallise as a hydrate, however the ratio of hydrate was variable and difficult to control. The compound was dissolved in five water miscible solvents and evaporated. DSC data (Figure 3) showed that is was possible to displace the hydrate with an alcohol, and whilst the form was now a solvate, it opened up opportunities to progress this compound.

Figure 3: DSC analysis showing displacement of hydrate



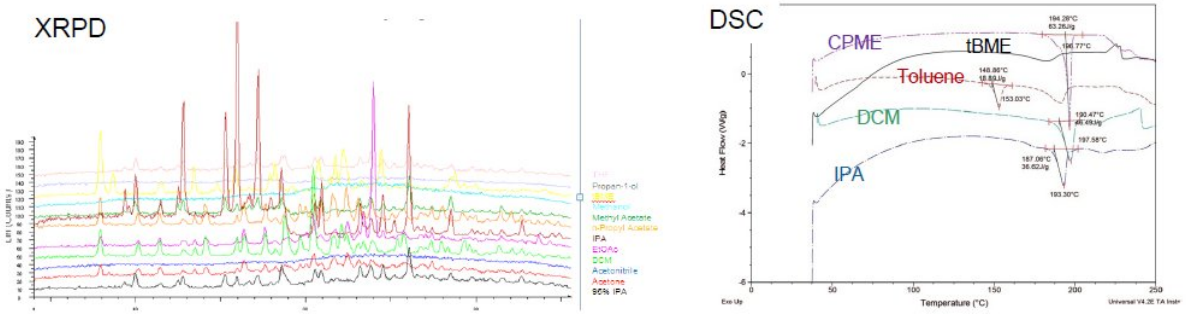
Another ongoing project had produced a compound with two crystalline forms, one of which was unstable. eXalt was used to recrystallise the compound from 16 different solvents over 96 hours. 11 produced crystals that were confirmed by XRPD, 10 were sent for single crystal x-ray (SCXR) for analysis, and 7 of those were found to be high quality crystals. This established a wide range of conditions for working up methodology and had produced seed crystals which could also be used to control morphic form.

Another ongoing project which had so far only produced amorphous compounds, had two leading candidates, B & C. These were taken and screened over 72 hours from 20 different solvents. Compound B produced crystals from three solvents. This data was then successfully utilised to identify two of these solvents to scale-up crystal production from 5mg in eXalt to 200mg using the crystals formed as seeds in the process. Compound C produced two hits which were oily and of poor quality, requiring further work. Subsequently Compound C was deprioritised as B was taken forward in the project.

Another ongoing project with 4 candidate Compounds, A, B, C & D, all in amorphous form and had never been crystallised before. More of Compound A & B was available, and this was screened in 18 and 20 solvents (respectively) using eXalt. A produced crystals from 10 solvents (Photo C), and B from only 2. These data were used to help guide selection of solvents for studies with C &D where material was less abundant. C & D was screened in 9 and 8 solvents (respectively) and crystals of C were obtained from 7 solvents and D from 5.

Photo C: Compound A crystallised from cyclopentylmethylether

Figure 4: Crystals of Compound A Analysed by XRPD & DSC



Of the 10 hits for Compound A, 8 were confirmed by XRPD, and 5 by DSC (Figure 4). The crystal from Toluene (Photo D) was a wet oil at the end of evaporation and grew to form an excellent single 5mg crystal on standing. 5 of the solvents were repeated, including toluene, where it was fully dried in the system.

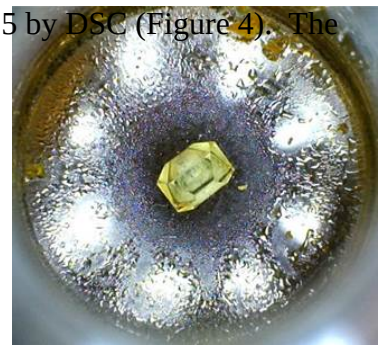


Photo D: Compound A crystallised from Toluene

In general, solvents which were successful for compound A were also successful for compounds C and D. This may not be surprising as all the compounds had structural similarities. However, this was not always the case and in one instance a solvent which was unsuccessful for both A and B was successful for C. Similarly another solvent which was unsuccessful for both A and B was successful for D. It is, therefore, desirable to screen as many solvents as practical for each test compound.

Summary

- eXalt was used to successfully crystallise 8 compounds from 4 projects, 6 were crystallised for the first time, 2 require more work.
- Limited material was required, approximately 5mg per vial was sufficient, allowing a screen to be run with as little as 50mg. The crystals formed were sufficient to produce enough material for characterisation by XRPD, DSC and SCXR and provide seed crystals for follow-up investigations. The technology is non-destructive, in that you can recover all of the poor quality crystals and amorphous test material.
- Expanding the solvent list supplied from Genevac was not successful in that it is not intuitive to calculate the settings, and it seems this needs to be done empirically. Some crystals were successfully formed from some of new solvents that were trialled, however the baffles and evaporation times were probably sub-optimal.

Opportunities for the technology seem to be that it could configured to be open access and available to all chemists running late stage research projects. It can be used with limited material and with confidence. Most success was had with crystallising amorphous compounds that had not been crystallised before. Less success was had with polymorph production, solvates and co-crystal formation, in the latter cases due in part to time available.

Challenges would include the development of a more comprehensive solvent list and configuration menu for the baffles. In addition, the development of eXalt was found to put greatly increased pressure on downstream analytical processes, namely XRPD, DSC and SCXR, because a great number of crystals can be formed relatively quickly and easily.

Acknowledgements
This paper is a review of the presentation "eXalt™ Technology – User Perspective Feedback" by Julia Hatto from Novartis Pharmaceuticals Global Discovery Chemistry Group, Horsham, UK and Huw Evans, undergraduate student at University College London. The views expressed in this review paper are onlythose of the author and are not endorsed by Novartis Pharmaceuticals or University College London.

The figures shown are all kindly provided by Julia Hatto from Novartis Pharmaceuticals, with the exception of Figure 1.