



encapsulated nanodroplet crystallisation (ENaCt) for small organic molecules: polymorph discovery

Introduction

Crystallisation is of vital importance to the drug development pipeline of the pharmaceutical industry due to the existence of multiple crystal forms (e.g. cocrystals, solvates and polymorphs) of an active pharmaceutical ingredient (API). The physical properties of the crystal form of an API are important in manufacturing, API stability and can have significant impact on the rate of API uptake from the solid form (e.g. from a tablet), ultimately influencing both its formulation chemistry and the pharmacokinetics of the API.

The need to fully understand such properties has given rise to considerable interest in polymorph screening experiments, to identify the different crystal forms of an API as early as possible in the discovery pipeline or even guide synthesis strategies. The development of new high-throughput crystallisation techniques therefore has the potential to enable rapid characterisation of these different crystal forms.

Current methods for the crystallisation of small organic molecules (such as an API) typically employ milligrams of substrate (in μL volumes) per experiment, making experimental screening of large areas of crystallisation space impractical due to high costs in terms of time and sample material.

To overcome these limitations in polymorph screening, Encapsulated Nanodroplet Crystallisation (ENaCt) techniques have been developed, in which nanolitre scale crystallisation experiments are undertaken to directly generate suitable crystals for single-crystal X-ray diffraction (SC-XRD) analysis. Utilisation of single crystal diffraction techniques allows for detailed information on a new or existing polymorph to be easily obtained.

ENaCt allows hundreds of crystallisation experiments to be undertaken in a wide range of organic solvents, with only a few milligrams of total test substrate and with results within a few days, yielding a rapid and cost-effective method for polymorph screening of an API.¹

ENaCt uses nanolitre droplets containing the sample of interest dissolved in an organic solvent. The nanolitre scale of this technique minimises the consumption of test samples, ensuring that vastly more experiments can be run on any given sample. These nanodroplets are placed within an inert oil “encapsulating” the test solution, thus slowing the evaporative loss of the organic solvent allowing supersaturation and ultimately crystallisation to be controlled (figure 1).

key benefits

automated nanolitre scale crystallisation allows parallel experiments with multiple experimental conditions.

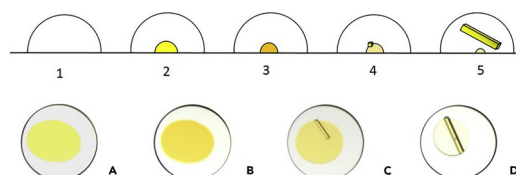
use of organic solvents

minimal sample use (nL)

rapid and cost-effective screening of crystallisation space

The SPT Labtech mosquito[®] provides the perfect platform for the development of ENaCt methods; the unique true-positive displacement pipettes allow accurate measurement of the nanolitres of solvent and oils required whilst being resistant to exposure to organic solvents. The SPT Labtech mosquito[®] can also handle liquids of varying viscosities – ideal for switching between different organic solvents and oils.

Figure 1. Cross-Section Schematic of an ENaCt Experiment (Top) and ENaCt Experiment with 200nL Mineral Oil and 50 mg/mL ROY in DMSO (Bottom). (1) Viscous inert oil dispensed onto a well of a 96-well glass plate, (2) solution of analyte in organic solvent injected into an oil droplet, (3) evaporative solvent loss to supersaturation, (4) nucleation, and (5) crystal growth. (A) solution of solvated analyte under oil, (B) evaporative solvent loss to supersaturation, (C) onset of crystal growth, and (D) complete crystallization.



Case study

Herein we demonstrate the efficacy of ENaCt for the screening of polymorphs, using 5-methyl-2-[(2-nitrophenyl)amino]-3 thiophenecarbonitrile as a test substrate, a synthetic precursor of the antipsychotic olanzapine. This molecule is also known as ROY because of the different colours (red, orange, and yellow) exhibited by its various crystalline states. ROY is known to have a large number of polymorphs and as is an ideal candidate for exploring the potential of ENaCt for polymorph screening.

Solutions of ROY were prepared in a wide range of organic solvents (e.g. acetone, ethyl acetate, ethanol, 1,2-dichloroethane, DMF, DMSO), near to the solubility limit. An SPT Labtech mosquito[®] liquid-handling robot was used to dispense 200 nL of an oil (FC 40, Fomblin YR, PDMSO, or mineral oil) into a 96 well SWISSCI LCP plate with a 100 micron spacer. Following this, 50 nL of each ROY solution was dispensed into each of the oil droplets within the wells. The LCP plates were sealed with a glass coverslip and stored in the dark at room temperature for 14 days.

Results

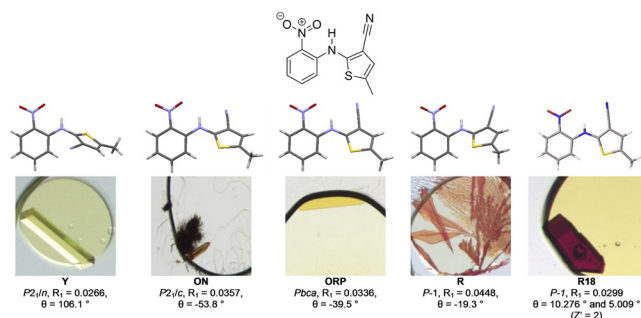
After 14 days, the 96 well LCP plate plates were examined by cross-polarising optical microscopy. Suitable single crystals of different polymorphs of ROY were obtained from a range of experimental conditions and were submitted for SC-XRD.

Conclusion

Encapsulated nanodroplet crystallisation (ENaCt) allows the rapid automated screening of crystallisation conditions for small molecules in organic solvents, with minimal sample requirements. The mosquito[®] is a key tool allowing the dispensing of the nanolitre droplets of organic solvent and inert oils required for this technique, with 96-well plate set-up times being as little as a few minutes.

ENaCt technology therefore provides the molecular sciences with a high-throughput tool to access single crystals of different polymorphs of small organic soluble molecules, which when combined with SC-XRD provides atomic level structural information on polymorphs formed. ENaCt has been further demonstrated as a tool for accessing crystal forms of a wide range of complex small molecules (bioactives, natural products, organometallics, etc.), providing structural and stereochemical information via SC-XRD, as well as acting as a novel polymorph screening tool.

Figure 2: Images of ROY crystals grown by ENaCt and associated single crystal X-ray diffraction data for Y, ON, ORP, R and R18 polymorphs.



References

A. R. Tyler, R. Ragbirsingh, C. J. McMonagle, P. G. Waddell, S. E. Heaps, J. W. Steed, P. Thaw, M. J. Hall, and M. R. Probert, *Chem*, 2020, 6, 1755-1769.

Further papers making use of ENaCt:

Zhu *et al.*, The Role of the Fused Ring in Bicyclic Triazolium Organocatalysts: Kinetic, X-ray, and DFT Insights., *J. Org. Chem.* 2022, 87, 4241-4253.

Al Subeh *et al.*, Structural Diversity of Perylenequinones Is Driven by Their Redox Behavior., *J. Org. Chem.* 2022, 87, 2697-2710.



Note on ENaCt usage

Licences for the commercial use of ENaCt technology (patent pending) are available on application, please contact Indicatrix Crystallography (www.indicatrix.co.uk) for further details. Academic, not for profit use of ENaCt is licence free.

