






Cite this: *CrystEngComm*, 2024, 26, 822

Developing a model-driven workflow for the digital design of small-scale batch cooling crystallisation with the antiviral lamivudine†

Thomas Pickles, ^a Chantal Mustoe, ^a Christopher Boyle, ^{ab}
Javier Cardona, ^{abc} Cameron J. Brown ^a and Alastair J. Florence^{*a}

We present a workflow that uses digital tools to optimise the experimental approach and maximise the efficiency in achieving the required process parameters for a desired set of crystallisation responses, kinetics and objectives. Model-driven small-scale experiments can contribute to reducing time and material waste in the development of pharmaceutical crystallisation processes. The workflow presented here guides the development of a small-scale batch cooling crystallisation process *via* solubility measurements, particle shape and size determination, form identification and preliminary kinetic parameter estimation to make crystals that satisfy quality target parameters (for shape, size and solubility) for a given active pharmaceutical ingredient (API). The case study herein follows the development of a crystallisation process for lamivudine, an API used in the preventative treatment of human immunodeficiency virus (HIV). This work identifies ethanol as a suitable solvent, meeting the acceptable solubility parameters for industrially relevant processes and yielded the biorelevant form, form I. The target kinetic parameters that were measured included induction time, growth rate and nucleation rate for lamivudine in ethanol under a range of conditions as guided by experimental planning models. Data was collected as part of the development of a DataFactory platform in which experimental optimisation can be autonomously implemented and all measurements stored in a crystallisation parameter database. This database will have further value in informing model development and continuous crystallisation process design and optimisation. The model objective-driven development workflow identified the following conditions, 19.9 °C, 600 RPM and supersaturation of 1.70, as achieving the desired objective successfully in 80 polythermal and 28 isothermal experiments. Integration of the workflow alongside the optimisation algorithm within the automated DataFactory system will enable fully autonomous, rapid data collection for small-scale API crystallisation. Such autonomous systems could play vital roles in pharmaceutical development and manufacturing driving towards more efficient and sustainable practices *via* digital transformation.

Received 11th September 2023,
Accepted 9th December 2023

DOI: 10.1039/d3ce00897e

rsc.li/crystengcomm

1. Introduction

Crystallisation is widely used as a fundamental purification step in the primary processing of active pharmaceutical ingredients (API) and many other molecular materials that also imparts the physical and bulk properties to the material relevant to subsequent processing.¹ The fundamental rate processes controlling crystallisation outcomes *i.e.* primary/

secondary nucleation, crystal growth, agglomeration, phase transformations, and impurity rejection can all be measured using a range of established techniques^{2,3} but remain difficult to predict for any given combination of API solute, solvent system, equipment geometry and process parameters. Hence process development can involve extended experimental efforts to select the composition and process conditions under which desirable outcomes can be achieved and transfer this across scales. Thus, approaches to enable rapid, optimised selection of conditions that provide the thermodynamic and kinetic control to achieve desirable size, shape and form outcomes early in the development cycle are of considerable interest.

Lamivudine, a nucleoside reverse transcriptase inhibitor used as an antiviral medication to treat and prevent human immunodeficiency virus (HIV) and hepatitis B virus (HBV),^{4,5} is also being investigated as a potential treatment for COVID-

^a CMAC Future Manufacturing Research Hub, Technology and Innovation Centre, The University of Strathclyde, Glasgow, G1 1RD, UK.

E-mail: alastair.florence@strath.ac.uk
^b Department of Chemical and Process Engineering, University of Strathclyde, Glasgow, G1 1XJ, UK

^c Department of Electronic and Electrical Engineering, University of Strathclyde, Glasgow, G1 1XW, UK

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d3ce00897e>


19.⁶ Lamivudine is currently produced using cooling crystallisation in methanol and (–) binol.⁷ There is limited prior literature describing the solubility of lamivudine and no kinetic parameters have been published. Past studies have shown that the dissolution rate of lamivudine is not substantially affected by changes in pH and that, subsequently, lamivudine exhibits good bioavailability in clinical usage.⁸ Good bioavailability is determined by the biopharmaceutical classification system (BCS) assignment of group III, noting it is on the border of group I for permeability.⁹ Lamivudine has been shown to produce three crystalline forms, form I, which is a 0.2 hydrate that exhibits needle geometry, and two anhydrous forms, form II, and III which typically adopt bipyramidal¹⁰ and needle morphologies, respectively.¹¹ Existing patents state that form I and form II are preferred in solid oral dosing.¹² Therefore, the objective of the crystallisation is the purification and management of physical attributes to ease downstream processing.

The DataFactory project at CMAC, The University of Strathclyde, involves the development of an autonomous data collection platform primarily focusing on API solubility and kinetic parameters.¹³ The work presented here lays the foundation for a robotic workflow that can be carried out without human supervision. The core experimental process will follow the steps discussed in this paper but be carried out robotically, *i.e.* by a Kuka KMR iiwa similar to the one used by Burger *et al.*¹⁴ When autonomous around-the-clock experiments are possible, the DataFactory will have the capacity to output data up to four times faster than human-led laboratory work due to operational hours increasing from 8–12 hours per weekday to 24 hours every day. A crystallisation parameter database is being developed to support the development of a suite of predictive tools in the form of a crystallisation classification system (CCS) that will accelerate the development of robust, sustainable crystallisation processes. This platform aligns with the wider need for more structured data management and curation adhering to findable, accessible, interoperable, and reusable (FAIR)¹⁵ data principles to support digital transformation in pharmaceutical and other process industries.

In this work, the target workflow builds on the stages and guidelines of previous process development workflows for pharmaceutical processes.² This workflow integrates predictive models, automation and robotics to improve overall experimental efficiency and speed. The workflow also establishes and tests the logic necessary to enable an autonomous robotic data-collection platform to undertake the key tasks towards the selected experimental objective. Such autonomous platforms have the potential to accelerate development timescales and allow rapid process design.¹⁶ Autonomous development systems that can intelligently vary experimental conditions to achieve a target outcome based on real-time data also promote sustainability by reducing human error, minimizing waste and optimising resource utilisation, contributing towards a more efficient and sustainable pharmaceutical manufacturing sector.¹⁷

2. Experimental

In this study, images taken during the crystallisation experiments were collected using Technobis's Crystalline Reactor system at a rate of one image every five seconds. Each of the eight reactor vials heats and cools following its own temperature profile, and every reactor has an HD camera focused on the lower part of the vial. X-ray powder diffraction (XRPD) patterns were collected using a Bruker D8 Discover, and the data was visualized using DIFFRAC.EVA¹⁸ software from Bruker. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed simultaneously using a Netzsch STA 449 F1 Jupiter and data was analysed using Proteus Analysis.¹⁹ Solid and solvent dosing was carried out using a Zinsser Crissy GGXXL robotic platform, which recorded solid and liquid dosed masses with a precision of ± 0.005 mg. The Zinsser platform was used to dose amounts in the range of 5 to 1000 mg of solid and 1 to 8 mL of solvent. Raman scans were collected using a Tornado Focused Non-Contact probe from a stirred suspension in 8 mL glass vials.

The optimisation of crystallisation parameters to achieve the desired outcome was conducted in Modde 12.1 (Sartorius)²⁰ following a full factorial design of experiment (DoE) plan with a multiple linear regression model (MLR). This model allows a 3-dimensional optimisation space *i.e.*, 2 objectives and a combination of input parameters. Additional optimisation objectives can be added as constraints but will not explicitly feature as objectives of the optimisation algorithm. The experimental section and subsequent optimisation were performed in tandem as batch offline iterations. The initial DoE plan had high exploration across the whole design space and covered the corners, edges and centre points. Iteratively, the DoE experimental coverage got smaller to focus on exploiting the true optimum. Termination criteria for the optimisation loop was ± 0.01 supersaturation and ± 1 °C between MLR recommended experiments.

3. Workflow

In this section, each stage of the workflow, corresponding to the boxes in Fig. 1, is described in detail. The challenges associated with the application of this workflow are also discussed for each step.

3.1. General considerations and challenges

The following section discusses the challenges associated with the generalisability of each step of the workflow. Each subsection corresponds to a step in the workflow.

3.1.1. Define the aim of the study. For the data collected using this workflow to be of use in the establishment of a CCS with broad applicability, the experiments need to span a varied chemical space in terms of physical and molecular descriptors. Consideration of scale and scope of experiments would benefit from standardisation so consistent



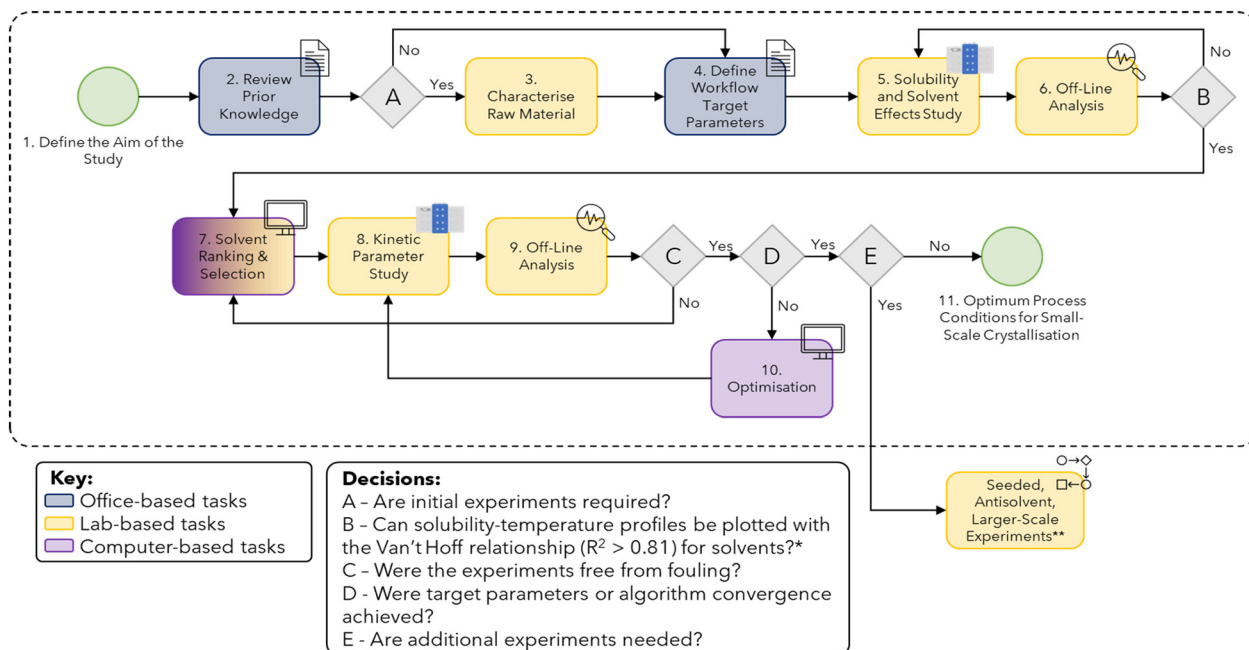


Fig. 1 Workflow for small-scale batch cooling crystallisation data collection (for more details refer to Table S1 in the ESI†). *Minimum of 3–4 data points must be used for reliable estimates of R^2 values. For some solvent systems, this may not be achievable as qualitative solubility can still be used to eliminate potential solvents. **Although not explicitly discussed in the case study of this paper, if the target parameters for the study are not met then further study can be done to explore seeded, antisolvent and larger-scale crystallisation.

information types and quality can inform future model development.

3.1.2. Review prior knowledge. Crystallisation and solubility databases are not freely available and/or do not present sufficiently consistent data across a wide enough chemical and process space, thus limiting their utility in this context. Reference solubility data may be very limited and/or difficult to gather. Furthermore, APIs passing through the workflow may be newly developed or legally embargoed and thus very limited prior data may be available particularly if this workflow was applied to a commercial setting. FAIR¹⁵ data principles should be adopted during data collection, storage and curation to enable the value of all data collected to be realised during and after the primary project.

3.1.3. Characterise raw material. It is important to understand the characteristics of the raw material before any processing to allow for referencing after crystallisation experiments have been conducted. Choosing the correct analytical methods for raw material analysis poses unique challenges depending on what equipment is available to the researcher.

3.1.4. Define workflow-specific target parameters. Target solubility,²¹ shape and size²² values can be estimated using developability²³ and manufacturability²⁴ considerations for different APIs. The target kinetic values, including nucleation and growth, needed to achieve these outcomes are not available without more detailed knowledge of the rate processes involved for each system. Another challenge associated with setting target parameters is determining the relative importance of each parameter – particularly when

introducing data filters and machine learning (ML) models. The relative importance of parameters is needed as these parameters will need to be weighted accordingly in multi-objective optimisation.

3.1.5. Solubility and solvent effects study (polythermal global search). Numerical values for solubility cannot always be measured due to equipment upper and lower constraints when dosing very high and low concentrations, *e.g.*, the lowest concentration dose possible may still not dissolve at the highest temperature. In this case, qualitative solubility observations, for example, either anti-solvent or too soluble, are sufficient for decision-making.

If prior solubility data is not available for a given system, initial experiments are conducted systematically or guided by predictive models.²⁵ When conducting experiments systematically, exploring concentrations at the lower and upper bounds of the target range allows us to generate qualitative solubility classifications that guide subsequent experimental concentrations. For some APIs, the workflow-specific target parameters may not be met using a single solvent from the library chosen. In this scenario, a binary solvent screen is recommended as intermolecular interactions in binary solvent systems can alter molecular solubilities.²⁶

3.1.6. Off-line analysis. XRPD is recommended as the main technique for polymorph determination as it is the gold standard for crystallisation polymorph fingerprinting and can identify amounts (typically down to 5%) of physical impurities.²⁷ An alternative and widely available fingerprinting technique for polymorph determination is



Raman spectroscopy which can provide information on solid form changes as well as yield. While both techniques are non-destructive, Raman can also be implemented *in situ via* optical probes removing the need for sampling. At the same time, the application of Raman can be limited by low signal-to-noise ratio and interference from solvent signal and fluorescence.²⁸ In some cases, the desired polymorph may not have been isolated at this stage. If this is the case, it may be necessary to revisit the solvent library and perform a wider form screen with the inclusion of binary solvent systems.

3.1.7. Solvent ranking & selection. This step is typically a manual human intervention step, and, therefore, poses challenges for autonomous implementation. Useful solvent selection tools are available to aid in this key decision step. These tools include, for example, the GSK Solvent Selection Guide 2009 (ref. 29) which classifies solvents as having one of the following: few issues, some issues or major issues (in terms of solvent favourability for use in industrial processes). These classifications are determined using physical properties, mainly melting point and boiling point, and a ranking of waste, environmental, health, safety, stability and life cycle impacts. The solvent ranking and selection step is important for the goal of the workflow as it can allow for the identification of a greener and easier-to-process solvent. Ultimately, experiments in this step could be limited to only those required for refining solubility predictions across a wide range of solvents for the API of interest.

3.1.8. Kinetic parameter study (isothermal local search). As the goal of this step is to determine estimates of the kinetic responses under representative process conditions, several approaches can be used to derive these from small-scale experiments.³⁰

The reliable measurement of the induction time of a solute in a given solution also requires multiple data points in order to properly sample the probability distribution.³⁰ The number of experiments and/or cycles required to address this challenge can therefore be expensive in terms of time and data storage. In this study, five cycles were used to reduce measurement uncertainty. Fewer cycles would have resulted in a higher degree of uncertainty while more cycles would have risked exceeding the computational and temporary data storage limits available for a given experiment.

3.1.9. Off-line analysis. Refer to section 3.1.6. If unexpected fouling has occurred at this stage, certain countermeasures such as altering heating rate, cooling rate, dissolution temperature and solvent choice can be explored.³¹

3.1.10. Optimisation. Selecting the optimal composition and operating conditions to achieve desired crystallisation outcomes with respect to yield, particle size, form and/or purity involves optimisation of several different factors and associated uncertainties. At this stage, it needs to be decided whether single or multi-objective optimisation will suffice for the aim of the workflow, as both come with separate challenges. Single factor, single response optimisation is

straightforward to implement but does not explore the impact of latent variables (such as how both temperature and supersaturation alter the growth rate simultaneously) or conflicting optimisation objectives (such as how induction time decreases as nucleation rate increases with supersaturation). On the other hand, in multi-objective optimisation, each target parameter could be an optimisation objective resulting in the challenges and complexities of a multi-dimensional optimisation problem. Going above 20 dimensions is often regarded as detrimental to many optimisation models.^{32,33} Multi-objective optimisation problems come with the additional requirement of data normalisation and the additional challenge of weighting target parameters appropriately to align to the aim of the workflow. If this optimisation approach does not achieve the desired shape, size and/or form objectives, then seeded-cooling and antisolvent crystallisation routes should be explored² (these methods are beyond the scope of the current case study and workflow).

4. Results & discussion: workflow case study of lamivudine

In this section the individual steps of the workflow, as described in Table S1 of the ESI,[†] are presented using lamivudine as a test case. Note, the section headings below, are numbered 4.2.X, where X refers to the specific stages of the workflow illustrated in Fig. 1.

4.1. Materials

Lamivudine (90 g, CAS ID: 134678-17-4) (Fig. 2) was purchased from Molekula Ltd as an off-white powder and form II. Lamivudine is a suspected teratogen. Therefore, dosing was performed in a fume hood and samples were transferred in capped vials. The solvents used for the cooling crystallisation were purchased from Sigma Aldrich, Fisher Scientific, VWR, and Alfa Aesar or prepared in-house (deionised water).

Lamivudine was chosen as the API of interest due to its useful pharmaceutical applications, and, in particular, its antiviral properties. There is also limited data in the literature for lamivudine solubility and crystallisation kinetics.³⁴ Contextual data was collated for lamivudine from

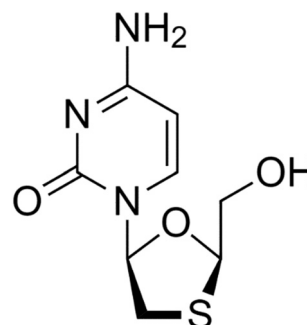


Fig. 2 Molecular structure of lamivudine.



the British National Formulary (BNF) and National Health Service of dictionary of medicines and devices (NHS dm+d).³⁵ The BNF recommends a dosage of 150 mg twice daily when used as a preventative treatment for HIV and HBV. The BNF and NHS dm+d shows current suppliers include Teva, Alliance and Milpharm. The developability classification²³ of lamivudine was calculated using BASF Zoomlab³⁶ and assigned a classification of group I, meaning that solubility and permeability are not expected to influence bioavailability.

The solvent library was chosen using the International Conference on Harmonization (ICH) residual solvent guidelines, safety, health and environment (SHE) ranking, cost, molecular descriptors, a principal component analysis (PCA) study in the literature³⁷ and a clusterSim study in the literature.³⁸ Relative locations of solvents on a PC1 vs. PC2 plot³⁷ of 272 solvents were used to ensure the diversity of solvents. To do this, solvents were selected from all four quadrants of the plot published by Diorazio *et al.* An online tool, from the same study, allows users to visualise solvents in different ICH and SHE classifications in their relevant quadrant of the PCA. ClusterSim³⁸ was then used to identify solvents with similar properties for our study. The solvents chosen from the PCA tool³⁷ were selected using a multidimensional scaling (MDS) plot which also showed similar solvents that sit within the same cluster.³⁸ While we ensured a diverse group of solvents was present in our selected solvents for this work, also choosing solvents from the same clusters allows us, and future users of the data provided, to compare experimental results within solvent families (*i.e.* alcohols) not just between solvent families to investigate any effects on crystallisation rate processes.

4.2. Key workflow tasks

4.2.1. Define the aim of the study. For this case study, the aim was to collect quantitative and qualitative solubility and solvent effects data and then subsequently optimise the small-scale batch cooling crystallisation process based on these results. The optimisation objectives were induction time and growth rate in response to supersaturation and isothermal temperature. Solvent choice in the optimisation was constrained with respect to solubility and shape and size parameters.

4.2.2. Review prior knowledge. Experimental data for lamivudine has previously been reported for a variety of techniques such as Raman,³⁹ IR,^{39,40} DSC/TGA,^{39,40} NMR,⁴ SEM⁴⁰ and XRPD.^{34,41} This data is used to validate our methods and confirm that the desired form is present. There are entries for each of the forms in the crystal structural database (CSD). The only lamivudine solubility publication available in the literature found by the authors was a study by Jozwiakowski *et al.* that showed that lamivudine has low solubility in most organic solvents except for ethanol, methanol and aqueous solvents.³⁴ The same study showed that crystallisation of lamivudine in methanol and water yielded needle habit.

Previous manufacturing routes consist of purification by crystallisation in methanol. This purification method allowed for the separation of the pharmaceutically active (–)-*cis* isomer.⁴²

4.2.A. Are initial experiments required? Yes, analysis of raw materials was required (see step 4.2.3 below for details).

4.2.3. Characterise raw material. The experimental DSC data for the lamivudine raw material showed a single peak at a melting endotherm of 178.6 °C (see Fig. S1 in the ESI†), and the TGA data showed no mass loss during heating (Fig. S2 in the ESI†). When compared to reference data, this data shows that the raw material is form II. XRPD also confirmed the presence of the most thermodynamically stable form, form II.¹¹ Later data collected in this study showed that form I, on the other hand, had a crystallisation exotherm at 139.1 °C (Fig. S1 in the ESI†) and melting endotherm at 178.8 °C with TGA data showing approximately 2% mass loss (Fig. S2 in the ESI†). This mass loss most likely corresponds to the removal of the 0.2 stoichiometric water molecule.

4.2.4. Define workflow specific target parameters. The target parameters for the solubility and solvent effects study were a lamivudine solubility of 0.005 g g^{–1} at low temperature (5–10 °C) and 0.05–0.25 g g^{–1} (ref. 21) at an elevated temperature (10 °C below boiling point) with an aspect ratio of above 0.5.²² These physical constraints were chosen to ensure a well-suspended slurry (*i.e.*, the elevated temperature concentration cannot be so high that a paste results at low temperatures), to promote a high crystallisation yield (*i.e.*, the low temperature concentration should be sufficiently low to crystallise most of the API but not so low to promote potential inclusion of impurities),²¹ and, finally, to encourage powder flowability of the resulting drug product (aspect ratio >0.5).²² At this stage of the workflow, yield and crystal shape are not optimised but, instead, used as constraints (*e.g.*, only solvent systems that give crystals with an aspect ratio exceeding 0.5 are accepted) to subsequently rank possible solvent choices.

The optimisation objectives of the kinetic parameter estimation had target values of an induction time of 1 hour and a growth rate of 1 μm min^{–1}. These target parameter values correspond to typical values that lead to operable conditions in larger-scale crystallisation processes.⁴³ Additional parameter constraints were included in the kinetic parameter estimation including a D90 size distribution of 100 μm to 250 μm so that the resultant powder would be free-flowing.²²

From a bioavailability and drug product perspective, there was no targeted preference between form I and form II as the both forms have similar oral bioavailability and are both used in commercial formulations.⁴⁴ Form I and form III exhibited needle geometry, and thus would not satisfy the aspect ratio target parameter. Form III is also currently not used as drug product. Form II, therefore, is the most desirable crystal due to increased thermal stability and flow properties for downstream processing.

4.2.5. Solubility and solvent effects study (polythermal global search). Known masses of lamivudine were weighed



into screw-top 8 mL vials with known volumes of solvent added automatically using the Zinsser Crissy platform, and a 10 mm PTFE magnetic stirrer bar was included in the vials prior to dosing. The vials were then placed in the Crystalline platform where each reactor had the following temperature profile applied:

1. Heat to 10 °C below the solvent boiling point or 90 °C (whichever is lowest) at a rate of 0.5 °C min⁻¹ and hold for 10 minutes.
2. Cool to 5 °C at a rate of 0.5 °C min⁻¹ and hold for 10 minutes.
3. Repeat the cycle 2 more times.

The slow heating rate in the above temperature profile was chosen to reduce the error of the dissolution temperature reported (refer to Fig. S3 in the ESI†). The maximum temperature was set to ensure that boiling solvents were not handled, thus making the experiment safe. The slow cooling rate was also used to reduce the error in determining the cloud point, and each sample vial underwent this

temperature cycle three times to check for anomalous results. The temperature profile was the same for all experiments and the stir rate was fixed at 600 RPM throughout.

The clear point, or the time at which full dissolution occurs, was defined as the temperature where no particles were observed in the collected images. In the literature, transmission data is used to identify the clear point.² Transmissivity, however, often gives a clear point below the true value as transmission reached 100% even when a few particles are still present in the images.

Similarly, the cloud point, or the time at which primary nucleation first occurs, was defined as the temperature at which particles were first observed in the collected images rather than when transmissivity goes below 100% or a similar threshold. The meta-stable zone width (MSZW) is the difference between the clear and cloud points as defined by the image data.

The solubility and solvent effects study determined that 11 of the 31 solvents were antisolvents (lamivudine solubility

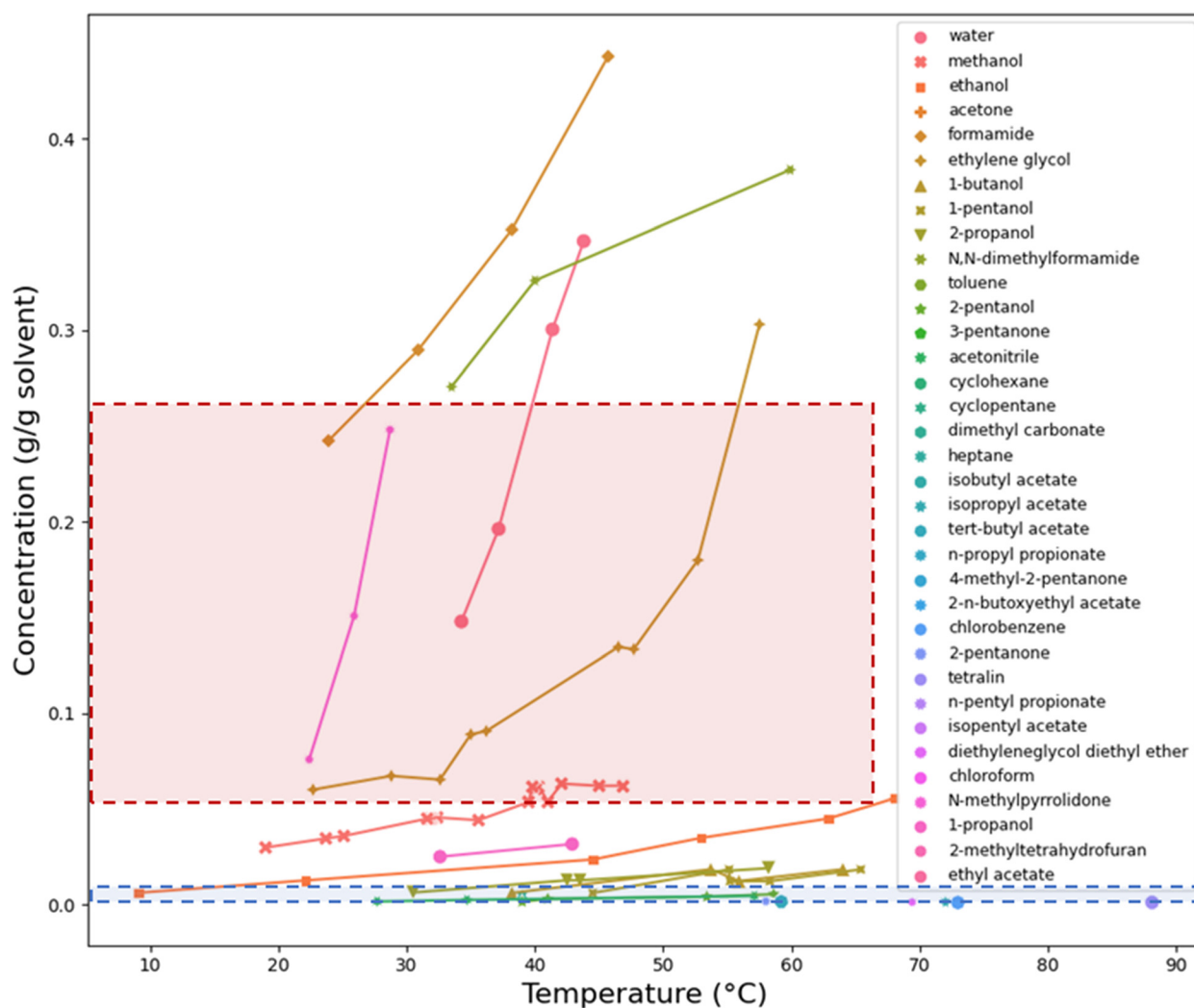


Fig. 3 The temperature-dependant solubility profile of lamivudine in 20 of the 31 solvents. The blue highlighted area shows solubility target parameters for low temperatures and the red highlighted area for high temperatures.



<0.005 g g⁻¹ at elevated temperature) due to dissolution not being observed for vials with 0.005 g g⁻¹ concentration of lamivudine. This concentration represents the lower limit of the Zinsser dosing platform's capabilities. The solubility profiles (Fig. 3) of lamivudine in the remaining 20 solvents showed that in 4 solvents, lamivudine was too soluble (lamivudine solubility >0.25 g g⁻¹ at elevated temperature), in 13 solvents, lamivudine was not sufficiently soluble (lamivudine solubility <0.005 g g⁻¹ at elevated temperature) and in the 3 remaining solvents, the solubility of lamivudine satisfied the solubility constraints. These 3 solvents were ethylene glycol, methanol and ethanol.

4.2.6. Off-line analysis. All powder product from the crystallisation experiments was filtered, and then XRPD was done to confirm which form was present. By comparing collected data to reference data, powder patterns (Fig. S4 in the ESI†) showed that form II recrystallised from ethanol and isopentyl acetate. 1-pentanol and chlorobenzene recrystallised only trace amounts, and large glass-like particles that diffract poorly grew in formamide precluding form identification. Crystallisation in all other solvents yielded form I, the undesired 0.2 hydrate form. The 0.2 stoichiometric amount of water in form I is likely attributed to residual water in solvents.

Small differences were observed in the powder patterns for the crystals grown in methanol, water and 1-pentanol. TGA and Raman was used to confirm that form I was present.

No major fouling or agglomeration was observed under the crystallisation conditions tested in any of the solvents used with the exception of 4-methyl-2-pentanone in which aggregates of needle crystals formed.

4.2.B. Can solubility-temperature profiles be plotted with the Van't Hoff relationship ($R^2 > 0.81$) for solvents? Yes, refer to Table S2 in the ESI†

4.2.7. Solvent ranking & selection. The 3 successful crystallisation solvents, in terms of solubility constraints (solubility of 0.005 g g⁻¹ at low temperature and 0.05–0.25 g g⁻¹ (ref. 21) at an elevated temperature), were methanol, ethanol and ethylene glycol. While crystallisation of lamivudine in ethylene glycol had a steep temperature dependence that would theoretically result in a high yield, ethylene glycol was still eliminated due to the solvent's high viscosity and consequently slow crystallisation kinetics. Nucleation was not observed for lamivudine in ethylene glycol despite cooling more than 55 °C below the dissolution point (*i.e.*, the supersaturation of lamivudine will greatly exceed the normal threshold for inducing nucleation). Methanol was also eliminated as crystals were form I with needle geometry, and the resulting aspect ratio did not satisfy the aspect ratio target value.

Ultimately out of 31 solvents evaluated for crystallisation of lamivudine, ethanol was the only system to satisfy the target parameters for shape and solubility. Specifically, these experiments showed that the form was II, the crystal shape was bipyramidal, and the solubility exceeded 50 g L⁻¹

at elevated temperature meeting all the objectives for this step.

4.2.8. Kinetic parameter study (isothermal local search) &

4.2.9. Off-line analysis. Similar to the solubility and solvent effects study, known masses of lamivudine were weighed into screw-top 8 mL vials with 2 mL volumes of ethanol added using the Zinsser platform, and a 10 mm PTFE magnetic stirrer bar was included in the vials prior to dosing. The solubility profile for lamivudine in ethanol from the solubility study was used to calculate the concentrations required for the relevant supersaturation. The vials were then placed in the Crystalline platform where each reactor had the following temperature profile:

1. Heat to 68 °C at a rate of 1 °C min⁻¹ and hold for 10 minutes.
2. Cool to the experimental temperature at a rate of 10 °C min⁻¹ with no stirring.
3. Hold at the isothermal point for 6 hours.
4. Repeat the cycle 2 more times.

The fast heating rate in the above temperature profile was chosen given that the dissolution point did not need to be measured; it was only necessary to ensure that dissolution was achieved. The maximum temperature was set at 68 °C to ensure that ethanol did not reach its boiling point and that solvent loss from evaporation was minimised. A fast crash cooling rate was used to achieve the isothermal temperature of interest as fast as possible to reduce the risk of nucleation occurring during the cooling ramp. The isothermal regime was held for 6 hours to capture crystallisations with longer induction times. Each sample vial underwent this temperature cycle three times to check for anomalous results and to get a median estimate for induction time. Induction times are reported as median values of these three measurements to reduce the impact of outliers. The temperature profile was the same for all experiments to minimise the variability across experiments. The stir rate was fixed at 600 RPM unless otherwise stated (refer to Fig. S5 in the ESI†).

Induction time, defined as the time between reaching isothermal conditions and the point at which crystals first start to nucleate, was determined using image data in this study, specifically using Helml's mean. Helml's mean⁴⁵ is an image feature used in computer vision that has been found to be a good indicator of the presence of particles in in-line microscopy.⁴⁶ Here, Helml's mean is calculated with a window size of 5 pixels and thus termed HELM₅. Induction time is estimated by measuring the time from temperature crash to where HELM₅ rises above a threshold. Due to differences in particle habit, lighting, and solubilities, the threshold is not a fixed value but is chosen based on the range of HELM₅ values obtained in each experiment. The threshold HELM₅ value is taken as the 5th percentile of the range of values HELM₅ obtained over the course of the experiment. Induction time estimation using HELM₅ has been shown to have improved accuracies over other approaches.^{46,47}



Finally, in this study image data was also used to determine the growth rate (the change in the particle size, over time). These parameters were determined using data spanning from slightly before the induction time (to account for potential errors in induction time estimate) until the time when the images became too crowded to identify single particles. Images were analysed to detect individual particles using the deep learning model “Mask R-CNN”.⁴⁸ This model was adept at identifying individual objects in crowded situations and at multiple scales making it well-suited to the task of particle characterisation.⁴⁹ Versions of the Mask R-CNN are available pre-trained on standard datasets which can be further trained (“fine-tuned”) to apply to new tasks. This transfer learning reduces the training time for new applications.

200 images from the Crystalline platform were chosen from various experiments to form a training set (which included both form I and form II of lamivudine amongst other APIs) based on their varied lighting conditions, particle habit, and particle solid loading. These images were manually annotated with annotations indicating the position and size of particles. These annotated images were supplied to a training algorithm to teach the Mask R-CNN model to detect particles on Crystalline images. The trained model was then applied to new images resulting in predicted particle locations, and a given confidence score. Predictions of confidence score less than 50% were discarded. The remaining particles were sized by fitting a rotated rectangle to the detected outline, minimising its area. The long side of this rectangle is referred to as particle length. Lengths were aggregated for an image and compiled in a histogram plot to determine the number-weighted-particle size distribution (PSD). Repeating across all images resulted in one PSD for every 5 seconds of the experiment during nucleation and growth. Quantiles and mean values of the PSDs were tracked over time. A linear fit was made to the mean size over time, and the gradient of the line gave an estimate of growth rate. The nucleation rate was estimated by tracking the number of detected particles for each image and again performing a linear fit of this number over time

with the gradient of the line giving the number of new particles per unit time.

An initial full factorial design screening experimental plan⁵⁰ was created (Table 1, “initial screening”). The experimental bounds on isothermal temperature and supersaturation ($SS = C/C^*$, where SS is the supersaturation, C is the solution concentration and C^* is the equilibrium concentration at the given temperature) were dictated only by physical limitations of solvent operating temperatures and industrial process solubility requirements to explore as large a design space as possible.

An MLR model was fitted to the initial results and the optimizer feature in Modde 12.1 was run with the stated target values for induction time (1 hour) and growth rate ($1 \mu\text{m min}^{-1}$). The MLR model trained on the initial screening results returned process conditions of a supersaturation of 1.70 and an isothermal temperature of 28.5 °C. Similarly, a partial least squares (PLS) model was fitted to the data where an optimisation returned similar values of a supersaturation of 1.69 and an isothermal temperature of 29.5 °C.

4.2.C. Were the experiments free from fouling? Yes, there was no significant fouling experimentally observed with ethanol as the chosen solvent system (visually observed in section 4.2.8).

4.2.D. Were target parameters or algorithm convergence achieved? No, there was no measurement at the MLR recommended supersaturation and temperature therefore experimental optimisation was required.

4.2.10. Optimisation. The subsequent (from the MLR model in section 4.2.8) full factorial experimental plans had a higher focus on exploitation over exploration, and, therefore, an increasingly narrower range was used for the supersaturation (± 0.27 to 0.09) and isothermal temperature (± 15 to 2 °C). The experimental plan followed by the fitting of the MLR model was done iteratively. As there was minimal change in supersaturation and temperature values predicted by the third and fourth MLR models (± 0.01 for supersaturation ± 1.0 °C for temperature), it was determined that convergence was achieved on the fourth MLR-trained model which returned process conditions of a supersaturation of 1.70 and a temperature of 19.9 °C.

Table 1 Process parameters and measured median induction time and mean growth rate for initial kinetic parameter screening of lamivudine in ethanol with a fitted MLR model. Standard deviations of 0.00 refer to where the image analysis only segmented one growth phase

Run order	Isothermal temperature (°C)	Supersaturation	Median induction time (s)	The standard deviation of induction time (s)	Mean growth rate ^a ($\mu\text{m s}^{-1}$)	The standard deviation of growth rate ($\mu\text{m s}^{-1}$)
Initial screening						
1	10	1.93	2778	374	0.0355	0.0052
2	25	1.67	3495	1073	0.0001	0.0213
3	25	1.56	3737	1496	0.0153	0.0200
4	40	1.84	1585	1555	0.0185	0.0173
5	10	1.42	11 380	2596	0.0263	0.0000
6	25	1.63	1591	1008	0.0287	0.0238
7	40	1.39	10 017	8011		

MLR output: SS 1.70, temp 28.5 °C

^a Growth rate values are missing due to the image analysis being unable to detect particle growth likely due to the small sample size of images collected at the point of nucleation before the images became too convoluted.



Table 2 Process parameters and measured median induction time and mean growth rate of kinetic parameter optimisation iterations of lamivudine in ethanol with fitted MLR models. Standard deviations of 0.00 refer to where the image analysis only segmented one growth phase

Run order	Isothermal temperature (°C)	Supersaturation	Median induction time (s)	The standard deviation of induction time (s)	Mean growth rate ^a (μm s ⁻¹)	The standard deviation of growth rate (μm s ⁻¹)
Optimisation iteration 1						
1	28.5	1.78	1524	81		
2	30	1.77	1310	248		
3	28.5	1.77	615	464	0.0186	0.0088
4	27	1.89	2000	374	0.0413	0.0309
5	28.5	1.8	2290	2023	0.0147	0.0000
6	30	1.68	4536	957	0.0740	0.0193
7	27	1.73	2460	895	0.0387	0.0000
MLR output: SS 1.74, temp 19.6 °C						
Optimisation iteration 2						
1	19.5	1.75	3901	301	0.0875	0.0778
2	18	1.74	1050	199	0.0440	0.0000
3	18	1.64	2305	3314	0.0472	0.0414
4	19.5	1.7	1745	564	0.0136	0.0196
5	21	1.81	2715	1483	0.0497	0.0265
6	21	1.68	6080	7293	0.0287	0.0000
7	19.5	1.72	1070	458	0.0279	0.0030
MLR output: SS 1.70, temp 19.5 °C						
Optimisation iteration 3						
1	21.5	1.68	2999	2132	0.0053	0.0014
2	21.5	1.62	2021	470	0.0015	0.0007
3	17.5	1.77	1986	803	0.0059	0.0005
4	19.5	1.72	1787	367	0.0028	0.0007
5	19.5	1.74	1506	986	0.0030	0.0018
6	17.5	1.6	11 552	2063	0.0020	0.0017
7	19.5	1.71	1320	3079	0.0089	0.0077
MLR output: SS 1.70, temp 19.9 °C						

^a Growth rate values are missing due to the image analysis being unable to detect particle growth likely due to the small sample size of images collected at the point of nucleation before the images became too convoluted.

All samples from each iteration of the optimisation loop were fingerprinted by XRPD (Fig. S6 in the ESI†) where results confirmed that all runs gave the desired form, form II. No fouling or aggregation was observed through image or observational analysis. The D90 of crystals detected by image analysis for the final run (run 7, optimisation iteration 3) was 105 μm. The aspect ratio was 0.62. Therefore, the kinetic estimation section of the workflow was completed as all target parameters achieved values within the designated range.

4.2.D. Were target parameters or algorithm convergence achieved? (Revisited). Yes, convergence was achieved as determined by the termination criteria.

4.2.E. Are additional experiments needed? No. A suitable process was developed using cooling crystallisation; therefore seeded, antisolvent and scaled-up experiments were not required to meet the objectives for this study.

4.2.11. Optimum process conditions for small-scale crystallisation. In this work, we have identified experimental conditions that yield lamivudine crystals with desirable physical attributes for pharmaceutical manufacture, specifically crystals with the biorelevant polymorphic form and an aspect ratio near 1 : 1. The process conditions required for these crystal attributes were isothermal crystallisation from ethanol at 19.9 °C, 600 RPM and supersaturation of

1.70. The resulting crystals had a D90 of 105 μm and a bipyramidal crystal habit. The solubility of lamivudine in ethanol also satisfied all target constraints with a value of 10 g L⁻¹ at low temperatures and a value greater than 50 g L⁻¹ at elevated temperatures. This approach also enabled kinetic parameter estimations for lamivudine from ethanol under various process conditions (Tables 1 and 2). These optimum conditions can be visualised in the DataFactory Dashboard (Fig. 4).

5. Conclusions

Following workflows such as the one presented in this work can provide direction and reduce cross-researcher variability in experimental work, thus promoting reliable, reproducible and robust data generation. This work has demonstrated the usefulness of the proposed workflow to go from API selection to an optimised system for small-scale batch cooling crystallisation. By following the workflow, we have determined an alternative method to the current crystallisation process of lamivudine, specifically recrystallisation from methanol that results in the more desirable bipyramidal habit of form II. This case study yielded experimental parameters for lamivudine crystallisation that satisfied our target parameters for an



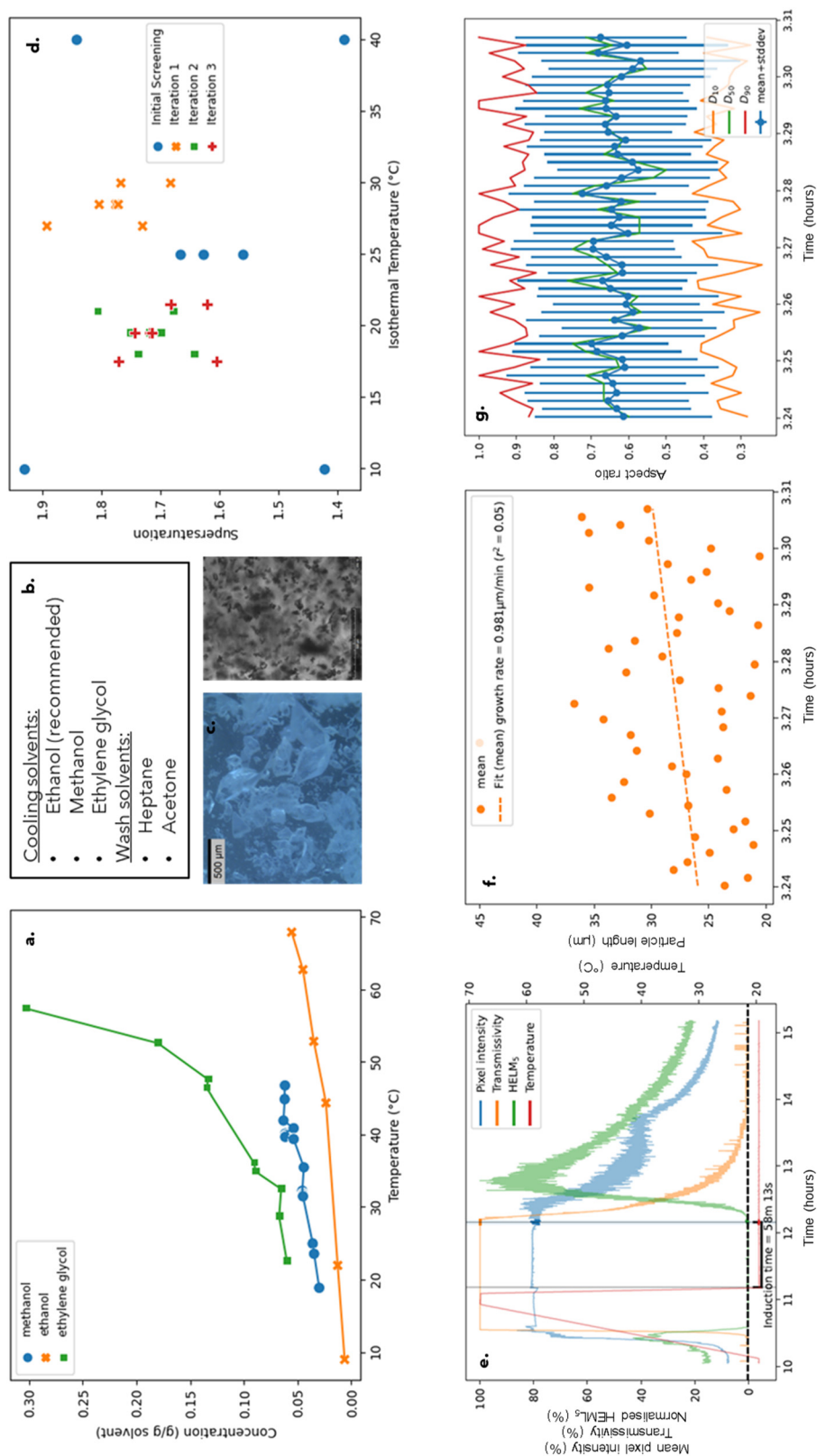


Fig. 4 DataFactory dashboard showing thermodynamic and kinetic crystallisation parameters for the proposed optimum lamivudine recrystallisation – solubility profiles (a), solvent classification (b), microscopy and crystalline images (c), DoE design space (d) and induction time (e), growth rate (f) and aspect ratio over time (g) for the optimum run.



operable isothermal crystallisation process from ethanol at 19.9 °C, 600 RPM and supersaturation of 1.70. Crystalline images and XRPD data confirmed that the resultant crystals were form II, the most stable form with desired downstream processing properties.

This work also shows that a solubility and kinetic dataset could be generated using only 53 g of lamivudine and 1169 mL of solvents compared to previous methods that used 252 g of solute and 1448 mL of solvents.² While less material was used in the work presented here, the reduction in material did not result in reduced information content as we collected approximately 7500 high-definition images with extracted thermodynamic and kinetic parameters and solution and solid-state data. Although droplet studies would result in significantly less material use, such thermodynamic and kinetic measurements would also not be possible. Furthermore, our contributions to the solubility and kinetic parameter literature data for lamivudine can also be used to inform future wet lab experiments and train and test improved solubility models.

Workflows such as this enable the future development of smarter experimental planning tools and predictive models, whereby model-driven experimental measurements can also be used to continuously improve models. Incorporating digital tools could help improve efficiency in crystallisation process development by reducing the amount of time and materials needed for the experimental sections of the workflow. This work has shown that the individual components, such as data analytics, optimisation and hardware, can be integrated and linked together in the logical, step-by-step manner necessary for achieving successful closed-loop automation.

This workflow can be applied to other APIs to identify industry-relevant, robust crystallisation conditions to inform the design of pharmaceutical manufacturing processes. Furthermore, applying this workflow to a wide range of APIs will enable the creation of a solubility and kinetics crystallisation parameter database that spans a varied chemical and crystallisation process space.

The availability of quantitative data on solubility and kinetic parameters in a wide range of APIs could aid laboratory-based researchers in optimising crystallisation processes to improve yield and crystal quality⁵¹ via the application of ML models.^{22,25,52} Such FAIR data collection for solubility and kinetic parameters can lead to the development of a crystallisation parameter database in a structured format developing benefits analogous to the CSD or the Protein Data Bank (PDB). Workflows such as this will allow us to generate the standardised data that will be an invaluable resource for the predictive design of molecular crystallisation processes.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank the EPSRC Future Continuous Manufacturing and Advanced Crystallization Research Hub (Grant ref.: EP/P006965/1) for funding this work. The authors would like to acknowledge that this work was carried out in the CMAC National Facility support by UKRPIF (UK Research Partnership Fund) award from the Higher Education Funding Council for England (HEFCE) (Grant ref.: HH13054) We are grateful to the Core Project MDPC industrial steering team: Jorge Calderon de Anda (AstraZeneca) and Francesca Perciballi (AstraZeneca), Neda Nazemifard (Takeda), Vaclav Svoboda (Pfizer), Chris Burcham (Eli Lilly), and Jan-Sebastiaan Uytersproot (UCB), as well as the observing members, especially Kevin Girard (Pfizer), for project guidance.

References

- 1 S. Vedantam and V. V. Ranade, *Sadhana*, 2013, **38**, 1287–1337.
- 2 C. J. Brown, T. McGlone, S. Yerdelen, V. Srirambhatla, F. Mabbott, R. Gurung, M. L. Briuglia, B. Ahmed, H. Polyzois, J. McGinty, F. Perciballi, D. Fysikopoulos, P. MacFhionnghaile, H. Siddique, V. Raval, T. S. Harrington, A. D. Vassileiou, M. Robertson, E. Prasad, A. Johnston, B. Johnston, A. Nordon, J. S. Srai, G. Halbert, J. H. ter Horst, C. J. Price, C. D. Rielly, J. Sefcik and A. J. Florence, *Mol. Syst. Des. Eng.*, 2018, **3**, 518–549.
- 3 S. G. Ring, P. Colonna, K. J. I'Anson, M. T. Kalichevsky, M. J. Miles, V. J. Morris and P. D. Orford, *Carbohydr. Res.*, 1987, **162**, 277–293.
- 4 R. K. Harris, R. R. Yeung, R. B. Lamont, R. W. Lancaster, S. M. Lynn and S. E. Staniforth, *J. Chem. Soc., Perkin Trans. 2*, 1997, 2653–2660.
- 5 D. T. Y. Lau and D. Lau, *Hepatology*, 2000, **32**, 828–834.
- 6 J. J. Garcia-Trejo, R. Ortega and M. Zarco-Zavala, *Front. Oncol.*, 2021, **11**, 664794.
- 7 B. N. Roy, G. P. Singh, D. Shrivastava, H. S. Jadhav, U. P. Aher and S. C. Deokar, An improved process for the manufacture of lamivudine, WO2011141805A2, 2011.
- 8 K. Prakash, P. Narayana Raju, K. Shanta Kumari and M. Lakshmi Narasu, *E-J. Chem.*, 2008, **5**, 1159–1164.
- 9 S. Strauch, E. Jantratid, J. B. Dressman, H. E. Junginger, S. Kopp, K. K. Midha, V. P. Shah, S. Stavchansky and D. M. Barends, *J. Pharm. Sci.*, 2011, **100**, 2054–2063.
- 10 R. K. Harris, R. R. Yeung, R. B. Lamont, R. W. Lancaster, S. M. Lynn and S. E. Staniforth, *Perkin Trans. 2*, 1997, 2653–2660.
- 11 R. Chadha, P. Arora and S. Bhandari, *Int. Scholarly Res. Not.*, 2012, **2012**, 671027.
- 12 B. P. Reddy, M. S. Reddy, P. V. Reddy and M. V. Kumari, Solid oral dosage forms of lamivudine, US8481554B2, 2009.
- 13 T. Pickles, C. Mustoe, C. Brown and A. Florence, 2022 APS Special Issue, 2022, 7.
- 14 B. Burger, P. M. Maffettone, V. V. Gusev, C. M. Aitchison, Y. Bai, X. Wang, X. Li, B. M. Alston, B. Li, R. Clowes, N.



- Rankin, B. Harris, R. S. Sprick and A. I. Cooper, *Nature*, 2020, **583**, 237–241.
- 15 M. D. Wilkinson, M. Dumontier, I. J. Aalbersberg, G. Appleton, M. Axton, A. Baak, N. Blomberg, J.-W. Boiten, L. B. Da Silva Santos, P. E. Bourne, J. Bouwman, A. J. Brookes, T. Clark, M. Crosas, I. Dillo, O. Dumon, S. Edmunds, C. T. Evelo, R. Finkers, A. Gonzalez-Beltran, A. J. G. Gray, P. Groth, C. Goble, J. S. Grethe, J. Heringa, P. A. C. 'T Hoen, R. Hooft, T. Kuhn, R. Kok, J. Kok, S. J. Lusher, M. E. Martone, A. Mons, A. L. Packer, B. Persson, P. Rocca-Serra, M. Roos, R. Van Schaik, S.-A. Sansone, E. Schultes, T. Sengstag, T. Slater, G. Strawn, M. A. Swertz, M. Thompson, J. Van Der Lei, E. Van Mulligen, J. Velterop, A. Waagmeester, P. Wittenburg, K. Wolstencroft, J. Zhao and B. Mons, *Sci. Data*, 2016, **3**, 160018.
 - 16 S. Knoll, C. E. Jusner, P. Sagmeister, J. D. Williams, C. A. Hone, M. Horn and C. O. Kappe, *React. Chem. Eng.*, 2022, **7**, 2375–2384.
 - 17 S. Guenat, P. Purnell, Z. G. Davies, M. Nawrath, L. C. Stringer, G. R. Babu, M. Balasubramanian, E. E. F. Ballantyne, B. K. Bylappa, B. Chen, P. De Jager, A. Del Prete, A. Di Nuovo, C. O. Ehi-Eromosele, M. Eskandari Torbaghan, K. L. Evans, M. Fraundorfer, W. Haouas, J. U. Izunobi, J. C. Jauregui-Correa, B. Y. Kaddouh, S. Lewycka, A. C. Macintosh, C. Mady, C. Maple, W. N. Mhired, R. K. Mohammed-Amin, O. C. Olawole, T. Oluseyi, C. Orfila, A. Ossola, M. Pfeifer, T. Pridmore, M. L. Rijal, C. C. Rega-Brodsky, I. D. Robertson, C. D. F. Rogers, C. Rougé, M. B. Rumaney, M. K. Seeletso, M. Z. Shaqura, L. M. Suresh, M. N. Sweeting, N. Taylor Buck, M. U. Ukwuru, T. Verbeek, H. Voss, Z. Wadud, X. Wang, N. Winn and M. Dallimer, *Nat. Commun.*, 2022, **13**, 3559.
 - 18 Bruker, *DIFFRAC.EVA: software to evaluate X-ray diffraction data (Version 5.2)*, <https://www.bruker.com/content/bruker/int/en/products-and-solutions/diffractometers-and-x-ray-microscopes/x-ray-diffractometers/diffrac-suite-software/diffrac-eva.html>.
 - 19 Netzsch, *Proteus*, (<https://analyzing-testing.netzsch.com/en/products/software/proteus>).
 - 20 Sartorius, *Modde version 12.1*, (<https://www.sartorius.com/en/products/process-analytical-technology/data-analytics-software/support/knowledge-base/modde-121-550674>).
 - 21 F. L. Muller, M. Fielding and S. Black, *Org. Process Res. Dev.*, 2009, **13**, 1315–1321.
 - 22 T. Deng, V. Garg, L. Pereira Diaz, D. Markl, C. Brown, A. Florence and M. S. A. Bradley, *Int. J. Pharm.*, 2022, **628**, 122309.
 - 23 J. M. Butler and J. B. Dressman, *J. Pharm. Sci.*, 2010, **99**, 4940–4954.
 - 24 M. Leane, K. Pitt and G. Reynolds, *Pharm. Dev. Technol.*, 2015, **20**, 12–21.
 - 25 A. D. Vassileiou, M. Robertson, B. G. Wareham, M. Soundaranathan, S. Ottoboni, A. J. Florence, T. Hartwig and B. F. Johnston, *Digital Discovery*, 2023, **2**, 356–367.
 - 26 J. Qiu, J. Albrecht and J. Janey, *Org. Process Res. Dev.*, 2019, **23**, 1343–1351.
 - 27 J. Boetker, T. Rades, J. Rantanen, A. Hawley and B. J. Boyd, *Mol. Pharmaceutics*, 2012, **9**, 2787–2791.
 - 28 T. Vankeirsbilck, A. Vercauteren, W. Baeyens, G. Van der Weken, F. Verpoort, G. Vergote and J. P. Remon, *TrAC, Trends Anal. Chem.*, 2002, **21**, 869–877.
 - 29 C. M. Alder, J. D. Hayler, R. K. Henderson, A. M. Redman, L. Shukla, L. E. Shuster and H. F. Sneddon, *Green Chem.*, 2016, **18**, 3879–3890.
 - 30 C. Brandel and J. H. Ter Horst, *Faraday Discuss.*, 2015, **179**, 199–214.
 - 31 M. Schoenitz, L. Grundemann, W. Augustin and S. Scholl, *Chem. Commun.*, 2015, **51**, 8213–8228.
 - 32 A. D. Clayton, E. O. Pyzer-Knapp, M. Purdie, M. F. Jones, A. Barthelme, J. Pavey, N. Kapur, T. W. Chamberlain, A. J. Blacker and R. A. Bourne, *Angew. Chem., Int. Ed.*, 2023, **62**(3), e202214511.
 - 33 R. Moriconi, M. P. Deisenroth and K. S. Sesh Kumar, *Mach. Learn.*, 2020, **109**, 1925–1943.
 - 34 M. J. Jozwiakowski, N.-A. T. Nguyen, J. M. Sisco and C. W. Spancake, *J. Pharm. Sci.*, 1996, **85**, 193–199.
 - 35 NHS, NHS (dm+d) (<https://services.nhsbsa.nhs.uk/dmd-browser/>).
 - 36 B. ZoomLab, https://www.basf.com/global/en/who-we-are/innovation/our-innovations/health_and_nutrition/zoomlab.html.
 - 37 L. J. Diorazio, D. R. J. Hose and N. K. Adlington, *Org. Process Res. Dev.*, 2016, **20**, 760–773.
 - 38 A. Johnston, R. Bhardwaj-Miglani, R. Gurung, A. D. Vassileiou, A. J. Florence and B. F. Johnston, *J. Chem. Inf. Model.*, 2017, **57**, 1807–1815.
 - 39 Y. Du, H. Zhang, J. Xue, W. Tang, H. Fang, Q. Zhang, Y. Li and Z. Hong, *Spectrochim. Acta, Part A*, 2015, **137**, 1158–1163.
 - 40 M. J. Jozwiakowski, N.-A. T. Nguyen, J. M. Sisco and C. W. Spancake, *J. Pharm. Sci.*, 1996, **85**, 193–199.
 - 41 S. J. Mazivila, R. A. E. Castro, J. M. M. Leitão and J. C. G. Esteves da Silva, *J. Pharm. Biomed. Anal.*, 2019, **169**, 235–244.
 - 42 B. N. Roy, G. P. Singh, D. Shrivastava, H. S. Jadhav, U. P. Aher and S. C. Deokar, An improved process for the manufacture of lamivudine, WO2011141805A2, 2011.
 - 43 D. A. Maldonado, A. Vassileiou, B. Johnston, A. J. Florence and C. J. Brown, *Digital Discovery*, 2022, **1**, 621–635.
 - 44 B. Parthasaradhi Reddy, M. Srinivas Reddy, P. Venkateswar Reddy and M. Vanaja Kumari, Solid oral dosage forms of lamivudine, US8481554B2, 2009.
 - 45 F. S. Helmlí and S. Scherer, Adaptive shape from focus with an error estimation in light microscopy, *ISPA 2001 Proceedings of the 2nd International Symposium on Image and Signal Processing and Analysis, In conjunction with 23rd International Conference on Information Technology Interfaces IEEE Cat.*, Pula, Croatia, 2001, pp. 188–193, DOI: [10.1109/ISPA.2001.938626](https://doi.org/10.1109/ISPA.2001.938626).
 - 46 F. J. S. Doerr, C. J. Brown and A. J. Florence, *Cryst. Growth Des.*, 2022, **22**, 2105–2116.
 - 47 T. Han, Y. Tang, X. Yang, Z. Lin, B. Zou and H. Feng, *Remote Sensing*, 2021, **13**, 4918.
 - 48 K. He, G. Gkioxari, P. Dollar and R. Girshick, Mask R-CNN, *arXiv*, 2018, preprint, arXiv:1703.06870, DOI: [10.48550/arXiv.1703.06870](https://doi.org/10.48550/arXiv.1703.06870).



- 49 C. Boyle, C. Brown, J. Sefcik and J. Cardona, *Improved Particle Characterisation from in-Line PAT: Comparison of Deep Learning and White-Box Methods*, Phoenix, Arizona, USA, 2022.
- 50 D. C. Montgomery, *Design and analysis of experiments*, John Wiley & sons, 2017.
- 51 H. Chen, R. H. Chiang and V. C. Storey, *Manag. Inf. Syst. Q.*, 2012, 1165–1188.
- 52 C. Xiouras, F. Cameli, G. L. Quilló, M. E. Kavousanakis, D. G. Vlachos and G. D. Stefanidis, *Chem. Rev.*, 2022, **122**, 13006–13042.

