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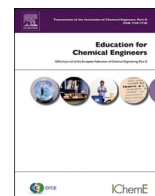
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Crystallisation: Solving crystal nucleation problem in the chemical engineering classroom based on the research grade experiments deployed in virtual mode

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ABSTRACT

Crystallization via nucleation can isolate active pharmaceutical ingredients from their crudes. While chemical engineering textbooks provide theoretical knowledge on crystallization and nucleation theories, they often fall short in providing practical insights on the nucleation mechanism. To bridge this gap, we introduced a virtual experiment on nucleation in second-year chemical engineering classrooms. The main goal is to educate students on crystallization procedures in research and process industries, teaching them how to analyse and manage collected data while integrating theoretical knowledge. This includes conveying the kind of information that can be obtained from a crystallisation process and instructing students on how to analyse and manage the data collected in the light of the theories learned. We devised an original chemical engineering problem on nucleation, derived directly from the raw data collected in the classroom from virtual experiments. This method differs from the conventional approach of solving standard textbook problems. The textbook problems, regrettably often lack crucial information on how nucleation rate or surface free energy are directly obtained from raw data. By the conclusion of the virtual experiment, students have acquired a comprehensive understanding encompassing both practical and theoretical aspects of crystallization, with a particular focus on nucleation. The methodologies elucidated in this study can be applied across a spectrum of chemical engineering modules, including process engineering, unit operations in chemical engineering, mass transfer, and can even be integrated into specialized courses dedicated to crystallization.

1. Introduction

1.1. Nucleation in chemical engineering classrooms: traditional approach

Crystallisation is one of the most important unit operations in chemical engineering (Crystallization, 2001; Mersmann, 2001). Crystallisation is widely used as a purification technique in food and pharmaceutical industries. One well known example in the food industry is the crystallisation of sucrose to remove sucrose from their impurities (Vasanth Kumar, 2010). In pharmaceutical industries, crystallisation is widely used to purify drugs and to produce crystals with desired polymorph (Chen et al., 2011; Kumar et al., 2021; Vasanth Kumar et al., 2023; Zhuang et al., 2023). Pure crystalline solids can be obtained from

an impure solution via nucleation or through controlled crystal growth. The driving force for crystallisation is supersaturation and depending on the level of supersaturation, compounds can be purified via nucleation or crystal growth. Typically, nucleation will be the choice, if the intention is to isolate the targeted compound in crystalline form from a highly crude product. Nucleation mostly likely occurs when the solution concentration is equal to or above the supersolubility concentration above the metastable zone width or in the so called labile zone (Crystallization, 2001). During nucleation only the target compound whose concentration is above or equal to supersolubility concentration will selectively and spontaneously assemble in an orderly fashion to form molecular clusters which will evolve into stable crystals leaving the impurities in solution. Crystal growth will be the choice if the objective is to produce

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crystals with desired size, shape, polymorph and size distribution (Zhuang et al., 2023). At industrial scale, nucleation is considered to be important and will be the preferred choice if the objective is to isolate a pure compound from its crude versions due to high yield per batch and productivity when compared to crystal growth. The topic of nucleation is interesting and important as the process of ordered assembling of atoms and molecules even occurs in the biological (e.g., kidney stone formation), geological environments (e.g., formation of minerals in the form of magma), and is one of the most important areas of modern science and technology. Clearly the education on this topic becomes important as we need skilled engineers with gained knowledge on the fundamentals of nucleation and crystallisation (Peerapen et al., 2022; De Yoreo et al., 2015; Kim et al., 2014).

Nucleation is a complex phenomenon due to the stochastic nature and the inherently multi-scale nature of the crystallisation process. The stochasticity of nucleation is mainly associated with the multiple mechanistic but random events that occur at molecular scale which also dictates the properties of the bulk material like purity and crystal structure. Theoretically, nucleation refers to the phase change (formation of solid phase) which occurs via the formation of stable nuclei that has a stable surface and a stable volume. According to the classical nucleation theory (CNT), a stable nucleus form via the assembly of atoms or molecules in orderly fashion which eventually evolve into a crystal nucleus (Alexandrov and Makoveeva, 2023). Alternatively, according to the two-step nucleation theory, the molecules in solution may assemble to form an intermediate phase called solute clusters, from which a probably a stable nucleus will evolve (Alexandrov and Makoveeva, 2023; Gebauer et al., 2014; Russo and Tanaka, 2016; Erdemir et al., 2009). The events associated with these mechanisms are completely random and this means the time-scale associated with the formation of stable nucleus is not deterministic.

In the undergraduate classrooms, nucleation is often explained from purely a theoretical standpoint and the topic of nucleation is discussed while learning about crystallisation and the crystallisation theories. The definition of nucleation, different types of nucleation like primary, secondary nucleation are mostly explained from purely theoretical standpoint using the information available in the classical textbooks (Crystallization, 2001; Sangwal, 2007). Mostly, the students will be

asked to understand the topic of nucleation via problem solving. The traditional approach is to ask the students to solve the problem of nucleation using CNT, where the nucleation rate is made available to the students (see the solved examples 1 & 2 in the Box 1A of the Supplementary Information) and the student's task is to calculate the surface free energy and the Gibbs free energy of nucleation or vice versa.

It must be acknowledged that nucleation is a complex phenomenon and is not a topic that can be easily understood solely by reading theory or through solving textbook problems. Solving textbook problems can help student to use analytical expression such as CNT to obtain the parameters like surface energy and radius of the critical nucleus (see the example 1 and 2 in Box 1A of the Supplementary Information) or how to use an analytical expression to obtain the nucleation rate (see the example 1 in Box 1A of the Supplementary Information). However, this approach will leave the students to speculate about the nature of the nucleation and the mechanisms involved. It will never provide students, (i) the opportunity to realise why nucleation is stochastic, (ii) the chance to learn the concept of the growth and induction time, (iii) the chance to understand the mathematical fact that the so-called nucleation rate, J , which appears in textbook problems is a number that captures the stochastic nature of the nucleation process. Based on our classroom experience, we realised that, even after solving the typical textbook problems that may be similar to the ones in Box 1A (see Supplementary information), the students have no clear vision about nucleation or how the solution will look like before nucleation, or at the point of nucleation and after nucleation. In fact, the students failed to have a clear vision about the unpredictability nature of nucleation and why it is claimed as a rare event. Our classroom experience together with the online quizzes conducted through Socrative and interactions with students during both lectures and tutorial sessions, we realised that explaining nucleation rates in isolation, without showing the experimental evidence of the stochastic nature of nucleation, or the experimental significance of induction time results in students memorizing the theoretical concepts. This approach encourages speculation about the process rather than developing a genuine understanding of the physics of nucleation, including its theories and underlying mechanisms.

Box 1

A model example problem was collaboratively crafted in the classroom with the active involvement of students. This problem is based on the data (see Table 2) collected from the virtual experiment, aiming to provide a practical application of the theoretical concepts discussed in class. Note: Through this exercise, students actively engage in the problem-solving process, reinforcing their understanding of the virtual experiment data.

Example 1: Utilize the information provided in Table 2 to perform the following calculations and analyses:

- Calculate the nucleation rate.
- What is the probability of nucleation when time $t = 100, 200, 1000, 3000$ and 5000 seconds.
- Gibbs free energy required to form a stable nucleus.
- Calculate the critical radius of the stable nuclei.
- Make suitable comments on the effect of supersaturation on the nucleation rate.
- Make any comment on the effect of molecular volume on nucleation.
- Make suitable comments on the actual experimental data based on your understanding about nucleation.

Solution:

The nucleation rate can be obtained from the expressions given in Eq. (2)

$$P(t) = 1 - e^{-JV(t-t_g)}$$

In the above expression, t_g is the time of growth observed in the first vial. As the experiments were performed with solution of volume 20 mL, the V in the above expression is equal to 20 mL. To solve the above expression and to predict the nucleation rate, J , we taught the students a non-linear regression analysis that can be performed using Microsoft Spreadsheet, Microsoft Excel.

The non-linear regression analysis relies on a trial-and-error method, where the error distribution between the experimentally obtained induction time and the induction time predicted using Eq. (2) is minimised by minimising the error function, sum of the errors squared. The excel sheet which is used in the classroom is provided as a supplementary file. In the supplementary file, we also explained the non-linear regression analysis method that we used in the classroom. In Fig. 3, we showed the plot of $P(t)$ versus t . Fig. 3 shows both the experimentally obtained t values and the ones predicted using the Eq. (2). Clearly Eq. (2) well predicts the experimental data and this is not surprising as the sum of the errors squared between the t obtained from experiments and predicted by Eq. (2) is almost closer to zero ($\sim 10^{-3}$ to 10^{-4}).

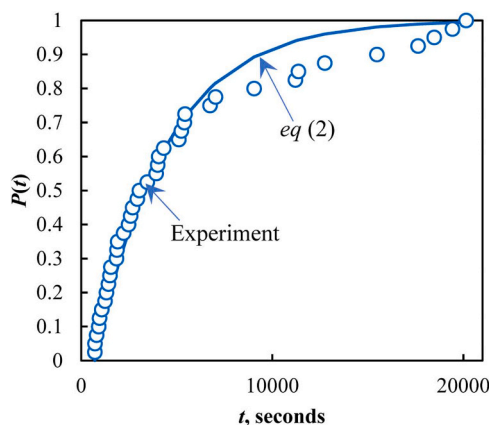


Fig. 3. Plot of the probability of nucleation versus induction time. We showed the data obtained from nucleation experiments (experimental data is given in Table 2) and the probability of nucleation predicted using the theoretical expression given in Eq. (2).

(a) From the non-linear regression analysis, we found that, the nucleation rate, J was found to be equal to 1.34×10^{-5} nuclei/20 mL.s or 40 nuclei/m³.min. (Note: We must mention to the students that the nucleation rate is usually an integer, and this refers to number of nuclei formed per unit time per unit volume of solution.)

Number of vials nucleated	$P(t) = M(t)/M$	t , seconds (experimental)	t , seconds (theoretical)	ERRSQ
1	0.025	708	0.000	6.67×10^{-3}
2	0.050	718	0.003	6.55×10^{-3}
3	0.075	804	0.025	5.70×10^{-3}
4	0.100	910	0.053	4.89×10^{-3}
5	0.125	952	0.063	4.63×10^{-3}
6	0.150	1068	0.092	4.02×10^{-3}
7	0.175	1240	0.133	3.33×10^{-3}
8	0.200	1314	0.150	3.09×10^{-3}
9	0.225	1421	0.174	2.79×10^{-3}
10	0.250	1497	0.190	2.59×10^{-3}
11	0.275	1547	0.201	2.47×10^{-3}
12	0.300	1842	0.262	1.85×10^{-3}
13	0.325	1864	0.266	1.81×10^{-3}
14	0.350	1904	0.274	1.73×10^{-3}
15	0.375	2213	0.332	1.22×10^{-3}
16	0.400	2471	0.376	8.57×10^{-4}
17	0.425	2583	0.395	7.18×10^{-4}
18	0.450	2684	0.411	6.03×10^{-4}
19	0.475	2934	0.449	3.60×10^{-4}
20	0.500	3042	0.465	2.75×10^{-4}
21	0.525	3451	0.520	5.65×10^{-5}
22	0.550	3914	0.576	5.86×10^{-6}
23	0.575	3995	0.585	1.69×10^{-5}
24	0.600	4052	0.591	2.79×10^{-5}
25	0.625	4317	0.619	1.13×10^{-4}
26	0.650	5102	0.691	6.22×10^{-4}
27	0.675	5231	0.702	7.33×10^{-4}
28	0.700	5394	0.715	8.80×10^{-4}
29	0.725	5431	0.717	9.14×10^{-4}
30	0.750	6739	0.801	2.19×10^{-3}
31	0.775	7024	0.816	2.46×10^{-3}
32	0.800	9045	0.893	3.76×10^{-3}
33	0.825	11204	0.940	4.02×10^{-3}
34	0.850	11365	0.942	4.00×10^{-3}
35	0.875	12745	0.960	3.73×10^{-3}
36	0.900	15472	0.981	2.87×10^{-3}
37	0.925	17624	0.989	2.19×10^{-3}
38	0.950	18472	0.991	1.95×10^{-3}
39	0.975	19425	0.993	1.70×10^{-3}
40	1.000	20142	0.994	1.53×10^{-3}
Σ ERRSQ				$99. \times 10^{-2}$

(b) Form the table, it can be observed that no vials are nucleated when $t = 100$ and 200 min. Thus, when $t \leq 200$, the probability of nucleation is zero. When $t = 1000$ seconds, at least 5 vials are nucleated. This means the probability of nucleation, $P(t) = M(t)/M = 5 \times 100/40 = 12.5$. When $t = 3000$ seconds, at least 19 out of 40 vials are nucleated and thus the chance of nucleation is 47.5 %. When $t = 5000$ seconds, at least 25 vials out of 40 vials are nucleated and thus the chance of nucleation is 62.5 %.

(c & d) To calculate the Gibbs free energy and the surface energy, it is essential to obtain the J at different supersaturation. If we have J at different supersaturation, then we can implement the classical nucleation theory (see Equation 9 in Box 3 A provided in the Appendix) to obtain the surface free energy. From the surface energy, we can calculate the Gibbs free energy at different supersaturation using eq (8) in Box 3 A (see Appendix). From the surface energy, we can calculate the critical radius of the stable nucleus using eq (6) in Box 3 A (see Appendix).

(e) According to the classical nucleation theory, explained in Box 3 A (see Appendix), the nucleation rate is inversely proportional to supersaturation. Thus, higher the supersaturation, higher is the chance of nucleation and nucleation rate.

(f) While the impact of molecular volume on nucleation hasn't been extensively addressed in lectures, it is understood that the Gibbs free energy necessary for stable nucleus formation (ΔG_c) exhibits an inverse proportionality to the square of molecular volume. In simpler terms, as the molecular volume increases, nucleation becomes more challenging. This correlation elucidates the challenges encountered in crystallizing larger molecules, such as proteins.

(g) From the table of $P(t)$ versus t clearly shows that (see the solution to part (a) of this problem), the chance of nucleation is stochastic. The point of nucleation varies from 708 to 20142 minutes and these events occur randomly and independently and this clearly exposes the stochastic behaviour of the nucleation. One safe conclusion that can be made is that higher the induction time higher is the chance for nucleation. However, this also depends on the type of API and the solvent involved, solution temperature and volume. As nucleation is a rare event, the chance of nucleation is higher in a solution of larger volume as it is more likely to experience this event in a solution of larger volume than in a solution of smaller volume. Thus, the table (see Table 2) generated based on the virtual experiment can take different numbers if we alter the solution volume, supersaturation, or the temperature.

METHOD 2: To calculate the nucleation rate

From the Table, it can be observed that 50 % of the vials nucleated when $t = 3042$ min. In that case, the nucleation rate can be calculated as follows:

$$J = 1/\tau_{50}.V = 1/(3042 \times 20) = 1.64 \times 10^{-5} \text{ nuclei/m}^3.\text{s} \text{ or } \sim 49 \text{ nuclei/m}^3.\text{min}$$

1.2. Why to deploy nucleation experiments in virtual mode?

Our classroom experience (discussed above) showed that the conventional approach failed to achieve the intended learning outcomes on the topic of nucleation. Thus, we took a more practical approach where we introduced the students the concept of nucleation by deploying a nucleation experiment in the classroom in virtual mode. As discussed in this work, deploying nucleation experiments in classrooms via virtual mode clearly achieved our intended learning outcomes and an opportunity to deploy research grade experiment in undergraduate chemical engineering classrooms. It helped the students: (i) how to conduct nucleation experiments as done in research labs, (ii) how the nucleation events inside the crystalliser are observed or captured, (iii) how the induction time are experimentally obtained, (iv) experimentally demonstrate the stochastic nature of nucleation, and (v) why nucleation is claimed to be a rare event. Additionally delivering the lectures on nucleation through virtual experiments helps to expose the importance of induction time and how it can help to calculate the nucleation rate. Teaching nucleation through virtual experiments allows us to demonstrate to students that nucleation is not deterministic. There is no unique value for the nucleation rate of a specific system; instead, we can only predict the probability of nucleation using a probability function. Additionally, as part of the learning outcomes, students will learn to interpret experimental data obtained from research-grade laboratories. They will implement crystallisation theories to predict important nucleation parameters. Unlike traditional textbook problems where nucleation rate or Gibbs free energy is provided, our approach involves having students document induction time by observing nucleation experiments. From these observations, students use nucleation theories to determine the crucial nucleation parameters. Additionally, as part of the learning outcome students will learn to interpret the experimental data obtained in research grade laboratories. can and implement crystallisation theories, to predict the important nucleation parameters. In contrast to solving traditional nucleation problems found in textbooks, resembling those in Box 1A (see Supplementary Information) where nucleation rate or Gibbs free energy is provided our approach involves

instructing students to document induction time by observing the nucleation experiments, from which the students used nucleation theories to obtain these important nucleation parameters. This particular approach enables us to effectively expose the probabilistic nature of the nucleation backed by evidence from experiments. The virtual nucleation experiment serves as a valuable tool for students to capture the importance of induction time, growth time, and nucleation from an experimental perspective. It provides a clear understanding and evidence that expose the random occurrence of nucleation events during experiments, allowing students to visually witness the evolution of the solution in the reactor before, during, and after nucleation. Furthermore, students gain insight into how experimental outcomes yield the probability of nucleation, influenced by one of the process variables such as supersaturation. Ultimately, the primary focus for students is learning how to extract the nucleation rate from raw experimental data. This skill becomes essential as they later apply classical nucleation theory to calculate parameters such as surface free energy, Gibbs free energy, or the critical free energy of nucleation. The breakdown of the topics delivered week by week with together with their intended learning outcomes will be discussed in detail in Section 3.

1.3. Practical limitations, required level of skill/experience for the instructors

It is important to note that nucleation experiments are both labour-intensive and time-consuming, demanding skilled personnel. Due to the stochastic nature of nucleation, its occurrence can span from minutes to hours, and in some instances, extend to days or weeks. This variability depends on factors such as the molecular volume of the crystallizing compound, as well as process conditions like initial supersaturation, temperature, agitation speed, and the volume of the solution. The choice of impellers or magnetic pellets for agitation also impacts nucleation. Consequently, executing a nearly flawless nucleation experiment necessitates considerable skill, meticulous planning, and precision, particularly during sample preparation, which can alone take

Table 1

Weekly teaching plan for executing nucleation experiments in virtual mode with intended learning outcomes. (Note: This teaching plan ensures a systematic approach to learn about the fundamentals of crystallisation, nucleation theories and knowledge on how to design nucleation experiments. This plan gradually builds the students understanding and skills week by week and provide the theoretical prerequisite to design or to understand the nucleation experiments. Additional support is also provided to students enrolled in this module through drop-in centres managed by the science learning centre, where students receive assistance from a tutor (one of the coauthors of this manuscript) for 2 hours per week for a minimum of 6–8 weeks leading up to the final exam).

Lectures/Tutorial topic	Intended learning outcome
<p>Week 1 Lecture hours: Crystallisation basics, applications in pharmaceuticals, ongoing research projects in lab, solubility, supersolubility, labile and metastable zones, purification via nucleation and crystal growth, supersaturation, MSZW, primary and secondary nucleation, polymorphism. Recommended reference materials: (Crystallization, 2001; Davey and Garside, 2000) Tutorial: Example problems on solubility, MSZW, and nucleation experiment design (see Examples 3 and 4 in the Supplementary Information). Quiz: Conducted during lecture hours (see Box 6 A of the Supplementary Information). Homework: Problems on solubility and MSZW (see Appendix Box 2).</p>	<p>Understand fundamentals and industrial importance of crystallisation. Design nucleation experiments using solubility and MSZW. Experimental methods to determine solubility and MSZW.</p>
<p>Week 2 Lecture hours: Classical and non-classical crystallisation. Nucleation mechanism observed under the transmission electron microscope. Gibbs free energy, classical nucleation theory. Definition of induction time. We defined our research problem: nucleation of salicylamide to achieve a desired polymorph, sharing solubility and MSZW data for salicylamide in isopropanol. Tutorial: We conducted virtual experiments on nucleation and collected experimental data. Additionally, we introduced nonlinear regression analysis and parameter estimation using Excel, with a model Excel sheet provided as a supplementary file.</p>	<p>Understanding crystallisation and nucleation theories is crucial for predicting parameters like surface free energy and the radius of critical nuclei, which are difficult to measure analytically. This knowledge is essential for interpreting data from virtual nucleation experiments. Additionally, advanced microscopy techniques help identify nucleation mechanisms, and understanding solubility and MSZW data is important. Students will develop skills to conduct nucleation experiments, collect relevant data, and analyze it using nonlinear regression analysis.</p>
<p>Week 3 Lecture hours: probability of nucleation, nucleation rate calculations using various methods from experimental data, and nonlinear regression analysis in Excel to predict nucleation rates (See Box 3 A of the Supplementary Information and the supplementary Excel spreadsheet). Tutorial: Solved additional exam-type problems where students calculate nucleation rates, Gibbs free energy, critical nucleus radius, and surface free energy without relying on computers (See Example 6 A in Box 7 A of the Supplementary Information).</p>	<p>Understanding the stochastic nature of nucleation and developing skills to solve problems using both computer-based non-linear regression analysis and traditional pen-and-paper methods to determine nucleation rates.</p>
<p>Week 4 Lecture/Tutorials: Crystal growth kinetics and pseudo second order expression (not discussed here). Additional support Furthermore, throughout the semester, we offered additional support to students enrolled in the Batch Process</p>	<p>Theoretical understanding on crystal growth kinetics. Understandings on the difference between purification via nucleation and only via crystal growth. To enhance student's problem-solving abilities and proficiency in deriving theoretical expressions, particularly focusing on topics relevant to</p>

Table 1 (continued)

Lectures/Tutorial topic	Intended learning outcome
<p>Engineering module from Week 3 to Week 12 through the Science Learning Centre (SLC). Students had the option to receive one-on-one or small group support at the SLC's drop-in centers (2 hours per week with dedicated tutoring).</p>	<p>nucleation experiments such as classical nucleation theory, nucleation rate, and Gibbs free energy.</p>

approximately 24 hours. Given the stochastic nature of nucleation, enhancing statistical reliability requires conducting multiple experiments. In such cases, the observed induction time in each experiment may vary from a few minutes to several hours or even days. Practically, incorporating nucleation experiments into regular undergraduate laboratory curricula poses a challenge, as these laboratories typically span no more than three hours. The nucleation experiments presented to chemical engineering students in virtual mode were originally performed by a fully trained and experienced PhD student (one of the authors, SK). Consequently, we contend that a virtual environment is more suitable for deploying nucleation experiments in classrooms. In this setting, experiments can be conducted by trained personnel or experts, and the recorded footage can be efficiently edited to convey the experimental outcomes to students within 30 minutes or less.

In this manuscript, we explained how the nucleation experiments were deployed in virtual mode in the chemical engineering classroom. Additionally, we provided a detailed breakdown on the lecture topics and tutorial contents delivered week by week and the learning outcomes from each week (see [Section 3](#)) that can be measured in terms of the gained theoretical knowledge and skillsets. We discussed about the required level of information/knowledge that should be acquired by the students from the lectures and tutorials before deploying the nucleation experiments. For the convenience of the instructors who may intend to deploy nucleation experiments in virtual mode, we provided our suggestions on what topics should be delivered and when it should be delivered before deploying the nucleation experiments. Additionally, we are providing two edited footages of the nucleation experiments performed at two different initial supersaturations and the data from the experimental outcomes (see the [Supplementary Information](#)). We also discussed about the tasks assigned to the students with respect to the nucleation experiments. We presented theoretical derivations of some important expressions (see Box 2 A and Box 3 A of the [Supplementary Information](#)) that cannot be easily found in the commonly used chemical engineering textbooks, original example problems that we created for students based on the nucleation experiments conducted in (virtual mode) the classrooms and from the data taken from the scientific literature.

It is worth mentioning here that we made the recorded video, along with the edited version of a model nucleation experiment, available to the general audience via YouTube. The explanations provided in the videos of the YouTube playlist can be used as reference material ([Vasanth Kumar; Vasanth Kumar](#)). These videos will serve as a guide for preparing and delivering lectures and conducting nucleation experiments in a virtual mode for students.

The rest of the manuscript and the information provided in [supplementary information](#) are written and presented in a way so that, it can be used as a reference material for both students and teachers who are learning or teaching the topic of crystallisation (nucleation and nucleation theories), to design tutorial problems, and even to create exam type questions. The manuscript and the [supplementary information](#) will stand on its own and it covers the fundamentals of nucleation that can be used to deliver teaching as research led topic on 'crystallisation in particular nucleation'.

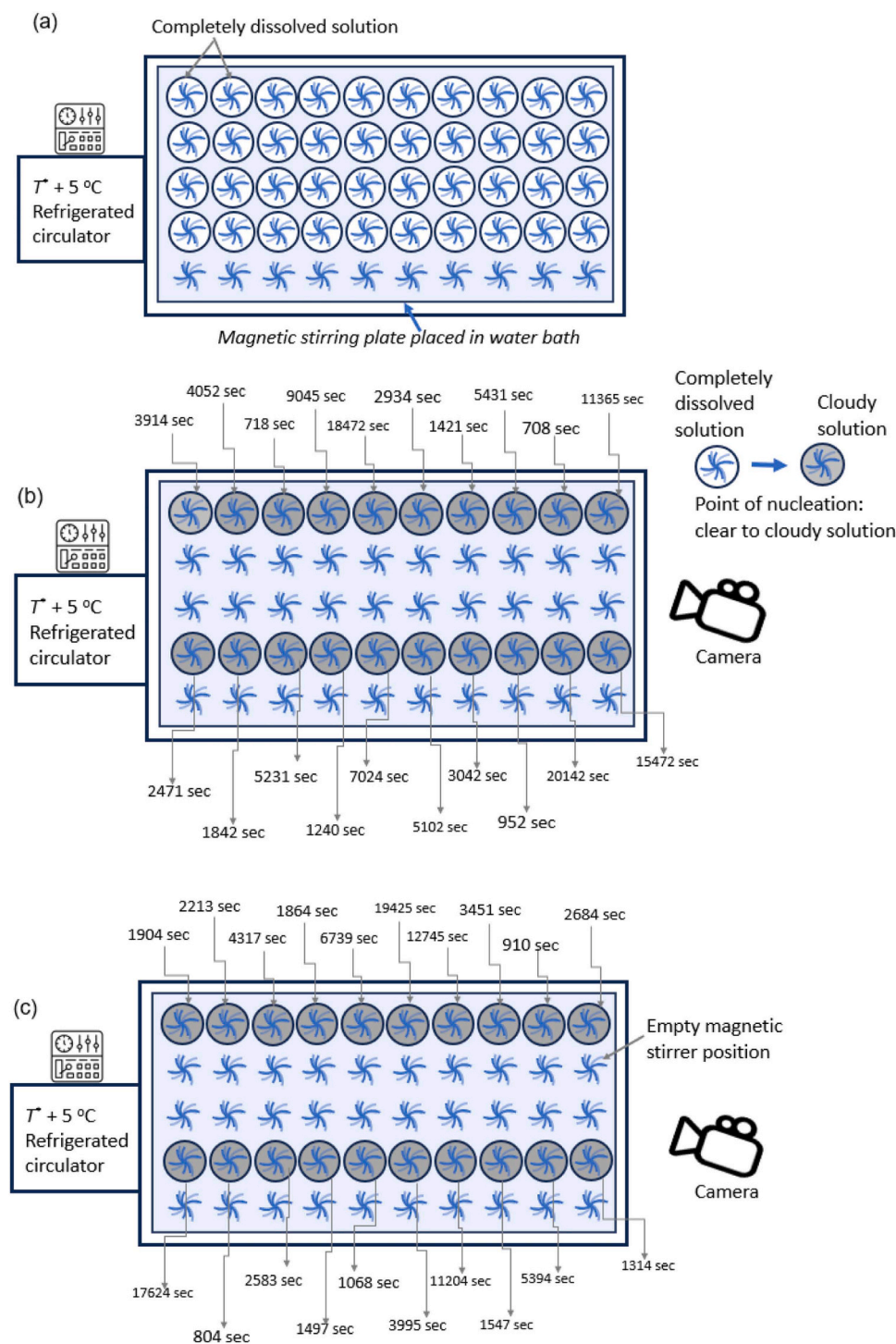


Fig. 1. The schematic illustrates the experimental set-up employed for nucleation experiments, utilizing a total of three water baths. (a) Equilibration bath: Maintained at a temperature of $T^* + 5\text{ }^\circ\text{C}$, this bath serves to prepare a fully dissolved undersaturated solution. (b and c) Nucleation baths: These two baths are maintained at the working temperature, T_w . The utilization of two nucleation baths was solely for the sake of convenience, enabling the clear observation of all vials through a digital camera positioned adjacent to the nucleation water baths. This setup ensures optimal visibility and efficient recording of the nucleation experiments, enhancing the overall practicality and effectiveness of the experimental process. To optimize visibility, it is essential to position the vials in a way that allows clear capture of the nucleation point using the digital camera placed adjacent to the water bath. We placed LED lights within the water bath to enhance the contrast for better observation. Figs. b and c showcase the induction time, capturing the moment of nucleation. Note to readers/instructors: These images were shared with students during the class (alternatively, we can provide the students just a table of induction versus number of vials nucleated), emphasizing the point of nucleation where the solution transitions from clear to a cloudy state. This visual representation enhances the students' understanding of the nucleation process, showcasing the changes occurring in each vial and providing valuable insights into the experimental outcomes.

2. The required level of theoretical knowledge for students

Before deploying the nucleation experiment in virtual mode, the students must have gained a theoretical knowledge and a general idea about the fundamentals of crystallisation, the driving force for crystallisation, solubility, metastable zone width, how to create supersaturation and how to exploit the solubility plot while designing nucleation experiment. For instance, we need the solubility of an active pharmaceutical ingredient in a specific solvent to create a supersaturated solution of that drug in a particular solvent. In the classroom, we explained how the supersaturated solution will be created and what type of information we need to create a supersaturated solution and in fact we solved few example problems (See the solved problems given in Examples 3 and 4 in Box 4 A of the [Supplementary Information](#)), which should give the students the essential knowledge and idea on how to prepare a supersaturated solution and to express supersaturation. Other key prerequisite is that the student must be aware of the solubility, the concept of metastable zone width, primary nucleation, and secondary nucleation, from a theoretical standpoint. For the instructors, the fundamentals of crystallisation, the concepts of nucleation, solubility, supersolubility, labile zone, stable zone, metastable zone and the metastable zone width can be found in the books that exclusively focus on the topic of crystallisation ([Crystallization, 2001](#); [Mersmann, 2001](#); [Davey and Garside, 2000](#)). Before deploying the nucleation experiments in the classroom, it is essential to introduce the students the concepts of nucleation, primary and secondary nucleation, why nucleation is important, and what happens in the solution at molecular scale during the nucleation. We believe that it is important for the students to have fundamental and theoretical knowledge on some aspects of crystallisation in particular nucleation and crystal growth. It is also essential to teach the students that the formation of the bulk crystals which we can observe with our naked eye in the crystalliser was preceded by molecular events which are not accessible to the human eye. We clearly explained the classical nucleation and two-step nucleation mechanism using cartoon images (See Box 5 A of the [Supplementary Information](#)). In the classroom, although we showed the mechanism of the two-step nucleation theory, we only discussed the theory that were originally developed to explain the phase change process involved in crystallisation via the classical process. We also mentioned to the students that, there is a big scientific debate going on in the scientific community regarding the actual mechanism of the nucleation. This lack of understanding on the nucleation process is currently driving the researchers from different disciplines that includes physicists, chemists, material scientists, computational chemists and chemical engineers make genuine efforts to provide or gain understanding on the nucleation process. As we are teaching to the undergraduate students, nucleation will be an ideal topic to showcase the students how researchers from different disciplines work together to bring an understanding of the process. In fact, in the classroom we showed some of the slides containing images generated by scientists who are using advanced scientific equipment such as TEM, AFM and in sit liquid cell TEM, and computational scientists that can expose the mechanism of nucleation of the process (these images are not shown here; however they can be found elsewhere) ([Davey and Garside, 2000](#); [Sleutel et al., 2014](#); [Fu et al., 2021](#); [Peng et al., 2014](#); [Nakamuro et al., 2021](#)).

3. Additional instructions for the teachers, weekly teaching plan and the intended learning outcomes

As part of this manuscript, we presented only the theories (Gibbs free energy and classical nucleation theory can be found in the Box 2 A of the supplementary file) that are required to exploit the data obtained from the virtual experiments. These theories allow to determine the parameters like interfacial tension, size of the critical radius which cannot be probed experimentally. However, to understand the experiment, the experimental outcome, data interpretation, requires a deep

understanding and knowledge on the fundamentals of the crystallisation. Thus, in the classroom, before deploying the nucleation experiment, we taught the fundamentals of crystallisation, driving force required for crystallisation (supersaturation), solubility, how to experimentally determine solubility, different units for solubility, how to define and calculate supersaturation, how to create supersaturation, how to express supersaturation, metastable zone width, how to experimentally measure metastable zone width and the importance of metastable zone width. Only then we explained the fundamentals of crystallisation, how molecules in supersaturated solution assemble to form solute clusters, how a crystalline structure may evolve from the solute clusters using cartoon images (see Figure 5 in Box 5 A of the [supplementary information](#)). In addition to these, during the lecture time we also showed some research works available in the literature. For instance, recently, a research group from Japan recorded for the first time the birth of nuclei under Transmission Electron Microscope. In the class we showed the TEM videos recorded by this research group to demonstrate, how nuclei can grow from a cluster like structure or dissolve back depending on the critical radius ([Nakamuro et al., 2021](#)). Then we explained the difference between the homogeneous nucleation, heterogeneous nucleation and secondary nucleation. Then we derived the Gibbs free energy (Please see Box2A in the [supplementary information](#)) and explained the classical nucleation theory (see Box 2A in the [supplementary information](#)). In total we spent 8 hours to deliver lectures on the fundamentals of crystallization, virtual experiment on nucleation and an experiment on crystal growth (which is not discussed in this manuscript). In these 8 hours we also solved several problems like the examples given in [Box 1](#), and the problems given in the [Supplementary information](#) (in addition to a problem that deals with the crystal growth of paracetamol that is not presented or discussed in this manuscript).

For the convenience of instructors who intend to deploy nucleation experiments in their classrooms, [Table 1](#) provides a comprehensive listing of topics delivered week by week to students as part of the 'crystallisation' and 'nucleation experiments deployed in virtual mode'. This table outlines specific topics covered during lecture and tutorial hours, along with the corresponding time allocated to each topic and the anticipated learning outcomes for students. For instructors intending to conduct nucleation experiments in a virtual environment, we strongly recommend following the sequence outlined in [Table 1](#) for delivering the topics. To help the instructors, who may benefit from this manuscript, we also provided useful reference materials in the [Table 1](#) in addition to the resources that are developed by us for the students. The arrangement of lecture topics is carefully structured for each week, ensuring that concepts learned in the current week facilitate understanding of topics to be covered in subsequent weeks. As indicated in [Table 1](#), with proper planning, the fundamentals of crystallisation and nucleation theories, followed by virtual experiments on nucleation, can be completed within three weeks. In terms of learning outcomes, while the primary goal of deploying nucleation experiments in virtual mode is to deepen understanding of the nucleation process, our materials are designed to enable students to acquire additional skills. These include the ability to analyse research data, develop spreadsheets for nonlinear regression analysis, interpret data considering theoretical models using non-linear regression analysis (will be discussed later in detail), and compare their results with those in the literature. To assist instructors, [Table 1](#) includes cross-links to the materials developed by us and presented within this manuscript, as well as references to external sources where high-quality scientific information can be obtained for developing lecture materials.

4. The nucleation experiment (deployed in the virtual mode)

Before presenting the video footage of the nucleation experiments to the students, we explicitly communicated that nucleation is a time-consuming process, necessitating multiple experiments to derive

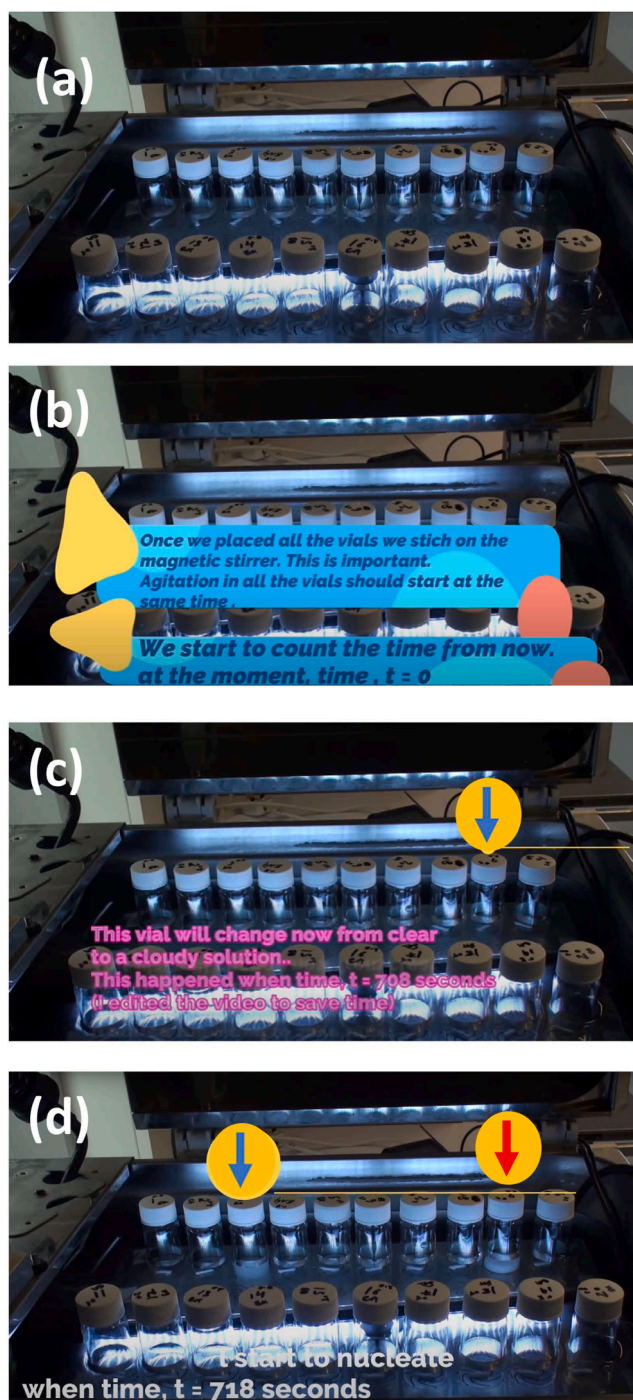


Fig. 2. provides a detailed breakdown of the nucleation experiment: (a) This snapshot displays the 20 vials positioned in the nucleation bath. Another nucleation bath, containing an additional 20 vials (not shown here), was also utilized. (b) The video footage imparts essential information to students. This specific snapshot underscores that the nucleation experiment commences when we activate the magnetic stirrer, initiating agitation. The initiation of agitation is set at the reference time, $t = 0$, to ensure experimental consistency. The scientific rationale is highlighted, emphasizing the sensitivity of nucleation to agitation. Consistent mixing conditions, commencing simultaneously, are crucial for accurate comparison across all vials. (c) This snapshot describes an experiment involving the observation of a phase change in a solution. Students are instructed to focus on a particular vial (indicated by a blue arrow), where they can witness a clear solution turning cloudy within seconds. The purpose of this observation is to demonstrate how once the first nucleus forms, millions of nuclei quickly develop in the vial, leading to a rapid transformation of the solution from clear to cloudy. This phenomenon highlights the kinetics of phase transitions and provides experimental evidence for the process. (d) The snapshot depicts a specific vial, denoted by a blue arrow, undergoing a transition from a clear to a cloudy solution at $t = 718$ seconds. Additionally, a red arrow points to another vial that underwent nucleation earlier, specifically at $t = 708$ seconds. This comparison highlights the difference in the timing of nucleation between the two vials and provides valuable insight into the kinetics of the phase transition process being observed. Note: Edited footage of a model nucleation experiment (performed with a solution of $S = 1.53$ is available as [supplementary information](#)). The edited footage of the experiment shown as snapshots in Fig. 2 can be found in the YouTube playlist ([Vasanth Kumar](#)).

Table 2

The results obtained from the nucleation experiments deployed in virtual mode. This table was generated using the edited video footage of the experiment and the data on induction time presented in Fig. 2b and c (please also refer to the work of Kakkar et al. (Kakkar et al., 2020)).

Number of vials nucleated	t, s	Number of vials nucleated	t, s	Number of vials nucleated	t, s	Number of vials nucleated	t, s
1	708	11	1547	21	3451	31	7024
2	718	12	1842	22	3914	32	9045
3	804	13	1864	23	3995	33	11204
4	910	14	1904	24	4052	34	11365
5	952	15	2213	25	4317	35	12745
6	1068	16	2471	26	5102	36	15472
7	1240	17	2583	27	5231	37	17624
8	1314	18	2684	28	5394	38	18472
9	1421	19	2934	29	5431	39	19425
10	1497	20	3042	30	6739	40	20142

statistically meaningful results. We underscored the non-deterministic nature of nucleation, emphasizing the unpredictability of experimental outcomes. Furthermore, we highlighted the susceptibility of nucleation to external factors such as impurities in the solution, fluctuations in temperature, and challenges in material handling and sample preparation. We also emphasized the importance of a skilled or, at the very least, a trained researcher to carefully execute a flawless nucleation experiment. In our class, we conducted experiments to explore the nucleation kinetics of salicylamide in ethyl acetate solvent. Salicylamide holds significance as an active pharmaceutical ingredient, renowned for its applications as an analgesic, antipyretic, and antiarthritic agent. During the session, we presented the students with experiments conducted at 15 °C, allowing us to calculate the nucleation rate as a function of supersaturation. At each level of supersaturation, we determined the nucleation rate based on the data collected from 40 individual nucleation experiments. The substantial number of experiments conducted proved to be adequate in capturing the stochastic nucleation behaviour of this system, enabling us to predict a statistically meaningful nucleation rate.

4.1. Experimental set-up and how the experiments were performed

The experimental setup for the nucleation experiments is straightforward, requiring three water baths equipped with magnetic stirring plates (see Fig. 1a to c). The first water bath, referred to as the dissolving bath, is used to dissolve crystals at an elevated temperature. The second bath, named the nucleation or working bath, facilitates the creation of supersaturation and serves as the site for conducting nucleation experiments. In the class, we specifically demonstrated the nucleation of salicylamide in ethyl acetate at a temperature of 15 °C.

To study the effect of supersaturation on nucleation kinetics, we deployed virtual experiments conducted at four different supersaturation. Up to 40 experiments were performed in parallel at each supersaturation at the nucleation temperature. Thus, in total, we performed up to $40 \times 4 = 160$ experiments for this virtual lab. For each experiment, we fixed the solution volume to 20 mL. The first step is to prepare a standard stock solution of salicylamide. A known mass of solute is dissolved in solvent at a temperature of 5 °C above the saturation temperature. The solute to solvent ratio should be fixed depending on the required level of supersaturation to be achieved at the nucleation temperature. For example, the experimentally obtained solubility of salicylamide at 15 °C is 90.41 g/L or 18.047 g/200 mL. To achieve a supersaturation ratio of 1.34 at 15 °C, it is essential to dissolve 121.14 g of salicylamide in 1 L of ethyl acetate or 24.18 g of salicylamide in 200 mL of ethyl acetate. The solution concentration of 24.18 g of salicylamide/200 mL of ethyl acetate solution is approximately equal to the solubility concentration at 28.2 °C (or in the other word the solubility temperature, $T^* = 28.2$ °C). This means, the salicylamide can be completely dissolved in EA at temperature, $T = 28.2 + 5$ °C = 33.2 °C. To ensure complete dissolution, we dissolved the API in the solvent

using a magnetic stirrer overnight at the elevated temperature ($T^* + 5$ °C). In the class we mentioned to the students that, we took a lot of care while performing the experiments and we tightly closed the reagent bottles to avoid solvent evaporation (As solvent evaporation will alter the solution concentration and thus the supersaturation ratio). We also mentioned to the students that we used a very clean reagent bottles to avoid any impurities entering the stock solution. For this virtual lab, we prepared $200 \times 4 = 800$ mL of stock solution, which should be sufficient to perform 40 nucleation experiments at each supersaturation.

For the nucleation experiments, 20 mL was pipetted out from the stock solution using a preheated syringe via a preheated 0.2 µm PTFE filters and transferred to the 30 mL vial. Following this procedure, we prepared exactly 40 vials. The PTFE filter was used to ensure the solution transferred into the vials are free of any foreign bodies. The PTFE filter and the syringe used to transfer the samples from the stock solution to the vials all are maintained at an elevated temperature to avoid temperature drop in the solution while transferring the solution from the stock solution (as a sudden drop in temperature while transferring the solution may trigger nucleation inside the syringe on the filters) to the vials. Then to each vial, we added a magnetic stirrer bar (Sigma–Aldrich, polygon shape, $1/2 \times 1/8$ in.) and then all the vials were tightly closed using a screw cap that has a PTFE seal. The vials were then placed again on the water bath which is maintained at a temperature of $T^* + 5$ °C. The solution was agitated at a constant agitation speed of 200 RPM overnight using the multi-position magnetic stirring plates placed inside in the water bath.

The next step is to perform the actual nucleation experiments. For this, we transferred the vials quickly from the dissolution bath (Fig. 1a) to the two nucleation baths (Fig. 1b and c) which are maintained at the working temperature. The agitation was again maintained at a constant agitation speed of 200 RPM using the multi-position magnetic stirring plates. Thus, the nucleation experiments performed in this work is slightly sophisticated and requires three isothermal water baths (Grant, GR150-S26 stirred with pump and has C2G cooling unit) that contains a multi-position magnetic stirring plates to provide agitation. In this work, we used two nucleation baths as we performed 20 nucleation experiments in each nucleation baths. Once all the 20 vials are transferred to the nucleation baths, we started the agitation and started to observe the vials for the nucleation using the digital camera. In the class, we mentioned to the students, that care must be taken while placing the vials on the bath and we should ensure that the digital camera focuses clearly all the 20 vials placed in nucleation baths. We recorded the nucleation experiment for 24 hours and then the point of nucleation observed in the each of vials was later obtained from the recorded videos by visually monitoring the solution turbidity. In this experiment, the point of nucleation or the induction time corresponds the time at which the solution in the vials turns from a clear to a cloudy/turbid solution.

For the students, we presented a time-lapse video capturing a specific nucleation experiment conducted with a solution of initial supersaturation, $S = 1.34$. The video showcased the seamless transfer of vials from

the dissolution bath to two nucleation baths. In the edited footage, we included additional instructions to provide enhanced details about the experiment. Fig. 2a to d display selected snapshots extracted from the video, offering students a visual narrative of the experiment. Fig. 2a exhibits vials placed in the nucleation bath, maintained at the working temperature, $T_w = 15^\circ\text{C}$. Fig. 2b presents details we communicated to the students during the actual footage, highlighting the successful transfer of vials from the equilibration bath to the nucleation bath. We emphasized the importance of switching on (as mentioned in the Fig. 2b) the magnetic stirrer simultaneously for all 20 vials to ensure uniform mixing at the same instant. In Fig. 2a and b, the solution in the vials is transparent and this means there is no phase change or in other words no nucleation in these vials. The sole distinction between Fig. 2a and b is that Fig. 2b denotes the initiation of mixing at $t = 0$ when we activated the magnetic stirrer. At this point, we asked the students to make a note that, 0 vials nucleated when time, $t = 0$. Additionally, throughout the experiment, we incorporated numerous notes in the video for the students, detailing the effort invested in placing the vials on the magnetic stirrer to ensure thorough and uniform mixing in all of them. In the experiment footage, we also emphasized the importance of uniform mixing in all the vials, highlighting how mixing can impact the induction time. Fig. 2c contains a note directing students to the specific vial where nucleation can be observed in the next few seconds. Out of the 20 vials within the analysis window, the vial indicated by the blue arrow in Fig. 2c will undergo nucleation, and we asked to students to observe the solution's transition from clear to cloudy at $t = 708$ sec (additionally, refer to Fig. 2d for an image of this vial post-nucleation –this vial looks turbid due to nucleation). Students are required to record the nucleation times for each vial in the provided table, using the experiment footage. In the table the students are supposed to record as 1 vial nucleated when $t = 708$ seconds. In Fig. 2d, we showed the nucleation of two vials. The first one nucleated when $t = 708$ seconds and the second vial nucleated when $t = 718$ seconds.

Throughout the edited footage, various notes were incorporated to provide information on the number of nucleated vials as a function of time, offering students a comprehensive understanding of the nucleation process and its dynamics. With an analysis window containing 20 vials, arrows are consistently added to indicate vials where students can expect to observe a phase change in the upcoming seconds, along with the time of nucleation in each vial. By indicating which vial to observe for the next phase change or nucleation event, students can efficiently track the progression of the experiment (this is essential as the students have no previous experience with nucleation experiments and it is a must to direct them using such indications and guide them where to monitor or observe the phase change events occurring in each of the vials. To assist the instructors who may intend to deploy the nucleation experiments in their classrooms, we are uploading two edited footages of the experiments performed at two supersaturations, $S = 1.53$ and $S = 1.76$. In the edited footage we also included some instructions that may benefit the instructors and the students.

During the experiment, it is the job of instructors to make the students realised that the solution takes some time which is equivalent to the induction time to form the first stable nuclei. However, once the first nucleus is formed via primary nucleation, it can immediately trigger the secondary nucleation making the solution to turn from clear to cloudy appearance in a split second. Based on this experimental evidence, it is easier to explain to the students the importance of the energetic barrier involved in the primary and secondary nucleation. For instance, once the first stable nucleus is formed it reduces the energy required to form the next stable nucleus and this triggers the birth of several nuclei in the highly supersaturated solution which is technically called as secondary nucleation. It is worth to mention to the students that, it becomes impossible to identify where the first nucleus is formed, and this is why we often call nucleation as a rare event. However, we also stressed the fact that, if we can perform nucleation experiment in a small volume, then it is possible to identify the nucleation spot, and this is something

that we should leave to advanced microscopic scientists who are performing nucleation experiments in nanolitres under microscope. While recording the data based on the virtual experiments, we also reminded to the students about the results from the group in Japan (Nakamura et al (Nakamuro et al., 2021).) that we shared with the students earlier during the lecture hours, where the scientists managed manages to capture the nucleation event under the microscope.

During the classroom session, we simplified the procedure for students. They were instructed to pay attention to the experiment's starting time, denoted as $t = 0$, corresponding to the moment when agitation commenced in the nucleation bath (refers the conditions in the Fig. 2b). All 40 vials were then transferred to the nucleation bath, and students were tasked with noting in their notebooks that $t = 0$ represented the onset of agitation. To expedite the process, we fast-forwarded the experiment videos in class since the primary focus was on recording the number of nucleated vials and the respective times of nucleation. Students were required to document the time at which they observed nucleation in each vial placed in the nucleation bath, considering a total of 40 nucleation experiments (or 40 vials). Their task was to create a table presenting the 'number of nucleated vials and the corresponding induction time'.

For instructors, we have prepared an activity sheet to be distributed to students in the classroom. This sheet will guide students in analysing experimental results described in accompanying videos. To optimize time utilization, it's crucial to showcase the moment of nucleation, ideally in at least three vials, along with the corresponding induction times, through edited video footage. Once students grasp the concept of phase change and how the solution rapidly transitions from clear to turbid via nucleation, instructors can provide Fig. 2b and c. Students can then use these figures to complete the remaining table detailing induction time versus the number of vials nucleated. The activity sheet, provided as a supplementary file, can be adjusted based on the number of experiments conducted.

For the convenience of the students, during the virtual experiment session, we presented two figures (see Fig. 2b and c) containing information about the point of nucleation or induction time observed in different vials. Table 2 was collaboratively created with the students in the classroom, incorporating data from the nucleation experiments conducted in virtual mode. This table includes information about the time at which each of the vials nucleated. Once the students are familiarised with the nucleation process and how the phase change looks like or appears in the vials, this image can be used to fill the table of the 'number of nucleated vials and the corresponding induction time'. The primary objective of deploying the nucleation experiment in virtual mode was to expose the stochastic nature of the nucleation process. The edited experimental footage and the information in Fig. 2b and c vividly demonstrate the achievement of this goal. Each vial nucleates at a different time, and there is no discernible trend between the number of vials nucleated and time. The randomness and unpredictability of the entire nucleation process are evident. For instance, if the first vial nucleates at 2 minutes and the second at 4 minutes, there is no rule dictating that the third vial should nucleate at 6 minutes. This randomness underscores the clear message we intend to convey to the students through this exercise. Additionally, we highlighted the futility of labelling vials as 1, 2, 3, and so on during nucleation experiments. Nucleation occurs randomly, irrespective of the vials' positions in the nucleation bath. The first vial labelled as 1 may end up being the last one to nucleate among the forty experiments at the studied supersaturation ($S = 1.34$). These key observations aim to emphasize the stochastic nature of the nucleation process to the students in the classroom. In the upcoming section, we will demonstrate how to calculate the nucleation rate and other essential parameters such as prenucleation factors and surface energy. We emphasized to the students the importance of paying attention to the number of vials nucleated at any instant of time during nucleation, rather than focusing on the numerical labels assigned to the vials. Fig. 2b, c, and Table 2 make it evident that nucleation is not

deterministic, and we can only predict the probability of nucleation. For instance, if one vial nucleates at time $t = 10$ minutes, the probability of nucleation when the time is equal to 10 minutes would be $(1/40) \times 100 = 2.5\%$. Similarly, if 20 vials nucleated at $t = 50$ minutes, the probability of nucleation at $t > 50$ minutes would be $(20 \times 100/40) = 50\%$. Likewise, if all 40 vials nucleate after 120 minutes, the probability of nucleation would be 100% when $t > 120$ minutes. This experiment clearly illustrates that nucleation is a more probabilistic event, and there is no unique value that can define the induction time and, consequently, the nucleation rate.

From the Table 2, it is possible to obtain the probability of the nucleation using the formula:

$$P(t) = M(t)/M \quad (1)$$

Where, M is the number of vials involved in the study or the number of experiments performed and $M(t)$ is the number of vials nucleated or the vials where we can observe or experimentally detect crystals at any time, t .

In the classroom, before showing the students how to calculate the nucleation rate from $P(t)$, we demonstrated to the students, the unpredictable nature of the nucleation process. It was emphasized that the nucleation rate we were about to calculate takes into account the stochastic nature of the nucleation process. As illustrated in the upcoming section, we employed a probability distribution function to derive the nucleation rate. We also mentioned to the students that, the accuracy of the nucleation rate that we are going to calculate can always be improved by improving their statistics which can be done by increasing the number of experiments. Higher the number of nucleation experiments performed, better will be the accuracy of the calculated nucleation rate. Additionally, we reiterated to the students the sensitivity of nucleation to supersaturation. Drawing from classical nucleation theory (see Box 2 A of the Supplementary Information), we highlighted that the nucleation rate is inversely proportional to supersaturation. This signifies that a higher supersaturation corresponds to an increased probability or likelihood of nucleation occurring. These insights aimed to deepen the students' understanding of the complex interplay between supersaturation and the probability of nucleation, enriching their grasp of the nucleation process.

5. Solving the nucleation problem

Before solving the nucleation problem, it is essential to derive the expression for the nucleation rate which is given in the Box 3 A (see Supplementary Information). It is important to show the students how the nucleation rate J is obtained from a probability function given by (See Box 4 A of the Supplementary Information for the complete derivation of Eq. (2)): (Jiang and Ter Horst, 2011)

$$P(t) = 1 - e^{-JV(t-t_0)} \quad (2)$$

In the classroom, after deriving the expression presented in Eq. (2), we guided students to apply their theoretical knowledge to solve Example 1, as outlined in Box 1 given below. Example 1 is created based on the actual experimental data presented in Table 1, offering students a practical application of the theoretical concepts discussed in class. This hands-on exercise serves to reinforce their understanding of the derived expression and its real-world application in problem-solving scenarios. This classroom exercise, detailed in Example 1 within Box 1, serves a dual purpose. It not only imparts essential knowledge about nucleation and crystallization theories but also underscores the practical relevance of these theories. On the mathematical front, students develop skills in analysing real-world data within the framework of theoretical models. The focus extends to gaining proficiency in data analysis, particularly through the execution of non-linear regression analysis using Microsoft Excel to determine nucleation parameters. A model excel sheet which we used to solve Example 1 given in Box 1 using non-linear regression

analysis is provided as supplementary file. In the excel worksheet, we also provided the instructions to obtain the nucleation rate, J using Eq. (2) using non-linear regression analysis. For nonlinear regression analysis, we used a trial-and-error method, to minimise the error difference between the $P(t)$ obtained from the experiments and $P(t)$ predicted using Eq. (2). The error distribution was minimised by minimising the error function, the sum of the squares errored between the experimental data and the ones obtained using Eq. (2) (see the Excel sheet provided as part of the supplementary information). Significantly, this exercise is designed with a research-oriented perspective, exposing students to the intricacies of handling research-grade data. More importantly, the students will also learn to implement nonlinear regression analysis to predict the theoretical parameters by fitting the experimental data in the theoretical expression given in Eq. (2). Beyond typical textbook content, which often simplifies crystallization as a chemical engineering unit operation, this exercise provides unique insights into how researchers address crystallization challenges, especially nucleation, and extract nucleation rates in laboratory settings. It bridges the gap between theoretical principles and their practical application, offering a more comprehensive understanding of crystallization parameters as obtained in industrial or research laboratory settings.

To address the problem in Example 1, as outlined in Box 1, access to a computer and suitable software for implementing non-linear regression analysis is evidently necessary. During the lectures, we emphasized to students that similar problem-solving scenarios could be expected in the final exam. Consequently, we introduced an alternative approach, denoted as Method 2 and detailed in Box 1 given below, to estimate the nucleation rate from the $P(t)$ versus t data. Method 2 operates on the assumption that the nucleation rate, J can be expressed as $J = N/V.t_{50}$, where t_{50} signifies the time at which the likelihood of nucleation reaches 50%. To elucidate, in the context of conducting 40 nucleation experiments, t_{50} would be the time when nucleation is observed in at least 20 vials. Method 2 can be used to solve Example 1 given in Box 1 without the assistance of any software or mathematical tools.

6. Additional problems

To enhance student's problem-solving skills and their understanding on nucleation and nucleation theories, we introduced several additional problems during classroom sessions. In Example 1, we calculated the nucleation rate at a fixed supersaturation. However, to obtain crucial thermodynamic parameters like Gibbs free energy and critical nucleus radius, necessary for employing classical nucleation theory, it's essential to determine the nucleation rate at different supersaturations. To illustrate this to students, we devised original example problems for them to apply classical nucleation theory in analysing experimentally collected data.

In Example 6 A, presented in Box 7 A of the supplementary information, we outlined a problem based on nucleation experiments conducted at different supersaturations. This problem tasked students with computing the nucleation rate at various supersaturations, utilizing Method 2 explained in Example 1 given in Box 1 for estimation purposes. By solving this problem, students could calculate theoretical parameters such as critical free energy and the radius of the stable nucleus, directly related to nucleation. Additionally, they learned how theories can assist in determining parameters that are difficult to probe using analytical techniques, such as the size of the critical radius. Furthermore, solving these additional problems served the dual purpose of preparing students for the final exam by exposing them to scenarios similar to those encountered in the upcoming assessment. Box 7 A (see Supplementary Information) also contains an additional problem relevant to nucleation (Example 6 A), along with its solution. These resources can be utilized by instructors who intend to deploy nucleation experiments in chemical engineering classrooms. For the final exam, 'exam questions' were developed based on information regarding the $P(t)$ versus t and nucleation rates of various active pharmaceutical ingredients (APIs) in

different solvents at different supersaturations, sourced from literature. Additionally, data obtained from our own lab was utilized. The model problems provided in Box 7 A will aid instructors in formulating exam questions or designing problems for tutorial sessions. To develop problems akin to those in Box 7 A, we suggest instructors to refer to experimental $P(t)$ versus t data for APIs in different solvents, which can be found in peer-reviewed research articles discussing the primary nucleation of various APIs. Such information is readily available in literature.

7. Student feedback, and their classroom experience

The incorporation of the topic of crystallization, along with virtual experiments on nucleation, was a significant aspect of the third-year chemical engineering curriculum within the Batch Process Engineering module. Three virtual experiments were introduced in this module, this includes covering nucleation (discussed in this submission), chromatographic separation of pharmaceutical compounds, and a separate virtual lab on Process Analytical Technology assisted crystal growth (to be submitted as a distinct work in the near future). The module designed based on the virtual experiments received exceptionally positive feedback, with a student's module evaluation score surpassing 4.8 out of 5. Students had a unique opportunity to engage with chemical engineering problems involving experimental data from research laboratories. Classroom interactions and attendance indicated high engagement during lectures and problem-solving sessions related to the virtual experiments. Many students expressed a keen interest in witnessing the actual experimental setups in the laboratory, and some even voluntarily requested to undertake their final year research projects on the purification of active pharmaceutical ingredients using crystallization, demonstrating genuine enthusiasm for the subject. Based on the module evaluation report, it appears that the majority of students found the lectures delivered based on virtual experiments to be both interesting and useful, with over 90 % expressing this sentiment. However, a smaller percentage, less than 10 %, found the problem-solving aspect of the virtual experiments to be quite intensive. This intensity seems to stem from the need to complete a classroom activity involving the creation of a dataset regarding the time versus the number of vials nucleated, followed by solving the nucleation problem using classical nucleation theories. This feedback suggests that while the lectures themselves were well-received, there may be room for adjustment or additional support for the problem-solving component to better accommodate students' needs and alleviate perceived intensity. The overall positive interactions with students during class sessions and in person meeting with the class representatives, coupled with the favourable student evaluation report, affirmed that students appreciate learning chemical engineering topics presented in a slightly unconventional manner through virtual experiments. They valued the chance to address a research-grade problem as part of their curriculum. Despite the success, it is important to acknowledge the requirement for skilled personnel to develop and execute a perfectly planned experiment that can be converted into a virtual experiment for deployment in the chemical engineering classroom. On the other hand, lectures developed based on virtual labs provide a valuable opportunity to bring works developed in research laboratories to chemical engineering classrooms. The module on crystallization and virtual experiments on nucleation was implemented successfully in the third-year chemical engineering classroom as part of the Batch Process Engineering module.

The module evaluation report also highlights that student experienced solving chemical engineering problems involving experimental data obtained from research laboratories for the first time. Despite this novelty, classroom interactions indicated high engagement during lectures and problem-solving sessions related to the virtual experiments. Many students expressed interest in observing the actual experimental setups in the laboratory, indicating a desire for hands-on experience. Furthermore, some students voluntarily sought to carry out their final year research projects on the purification of active pharmaceutical

ingredients using crystallization. Additionally, two students expressed their interest in pursuing a PhD on this topic, showcasing genuine enthusiasm and potential for future research in the field of crystallization and pharmaceutical engineering. Overall, these observations suggest that the module has successfully sparked curiosity and passion among students, encouraging further exploration and potential academic pursuits in related areas. One of the students who conducted their final year research project (refer to the report by Cliffe et al., (Cliffe, 2021)) on crystallization in our lab has successfully published a peer-reviewed research article (see Mayank et al., (Vashishtha et al., 2023)) on the topic of crystallization. This demonstrates the immediate impact of implementing research-grade experiments in the chemical engineering classroom. Solving research grade problems as part of the undergraduate curriculum inspires the undergraduate students and give them an opportunity to tackle a research problem and even to take a career decision. The positive interactions during class sessions, along with the favourable student evaluation report, confirmed that students appreciate learning chemical engineering topics in a slightly unconventional way through virtual experiments. They also expressed appreciation for the course materials, which encompass a research-grade problem addressing challenges encountered by the pharmaceutical industry and is integrated into their curriculum. Beyond student feedback, the understanding of the topic was tested using a Socratic quiz during classrooms. The class demonstrated full engagement, with over 80 % of the students participating in the quiz and providing correct answers to the questions. Model quiz questions used for this purpose are listed in Box 6.

8. Conclusions, additional thoughts and our classroom experience with students

To conclude, crystallisation and in particular nucleation is a complex process and is not easy to incorporate these topics as part of any chemical engineering laboratory exercise. Thus, it is best to deliver the lectures on these topics using a virtual lab. This manuscript details how these topics were delivered and how virtual experiments were conducted and deployed in chemical engineering classrooms. This approach not only kept the classroom more engaging but also provided students with an opportunity to solve a research-grade problem, understanding how industrial challenges are typically addressed in research laboratories. This skill set is crucial for undergraduate students as they are expected to encounter such problems in industries where they will be employed.

In our efforts to deploy nucleation experiments in classrooms, students were provided access to the same set of instructional videos. Each student watched these videos independently, recording their own induction times. They were then tasked with creating a table correlating time with the number of vials nucleated. This initiative marked our initial implementation of research laboratory experiments in chemical engineering classrooms. Given that students worked with identical experimental data, we required them to submit an honesty declaration as part of their assignment. However, if we decide to assign different experimental datasets in the future, we can still use the same set of experiment videos. In the present case, we have 40 experiments at each supersaturation. Regardless of the number of experiments involved, it is possible to ask the students to generate data with different dimensions. For example, it is possible to create a data of dimension $R \times C$ (i.e., t_{ind} x number of vials nucleated) based on the 40 nucleation experiments performed at each supersaturation; where R represent the rows that can range from 8 to 40 and number of columns will be equal to 2. Alternatively, as we did in the classroom, we can keep the task simple and ask the student to record the induction time observed in all the vials based on which they can generate a data of t_{ind} versus number of vials nucleated. Other approach can be to ask students to assume a fixed number of number of experiments, N that can range from 10 to 40 experiments or vials for each supersaturation. In that case students should

select any random but N number of experiments from the 40 experiments. From such a data of reduced dimension, it is possible to calculate the nucleation rate and other nucleation parameters. In this manner, each student will work with data of different dimensions but obtained from the same set of experiments. Nevertheless, it should be remembered that the dimensions of the nucleation parameters and the nucleation rate obtained from data of different dimensions of the same experiment may not vary significantly.

Once the students are familiar with the nucleation experiments, they can learn to interpret the experimental data obtained from nucleation experiments performed with a model system in the classroom. After this, we can provide them with experimental data for other systems without showing the actual footage of the experiments. For instance, it is always possible to generate a new database of induction time versus number of vials nucleated for different combination of APIs and solvents based on the information available in the literature. Based on the data obtained from literature, it is possible to cleverly generate charts similar to the ones shown in Fig. 2b and c. The charts should show the induction time of the vials and more importantly, the induction time should be randomly arranged to emphasise the stochastic nature of the nucleation. It should be remembered that the number of datapoints that we extract from literature can be flexible and the number of experiments at each supersaturation can be decided at the will of the instructor. Irrespective of the number of the experiments involved, the $P(N)$ can still be predicted. For e.g., for the final exam model questions, we created charts similar to the ones in Fig. 2b/2c that contains only five vials. Obviously, nucleation rate calculated based on the experimental outcome of five experiments might penalise the statistics, nevertheless such simple and small dataset helps to test the learning outcome of the students in the final exams that do not run for more than 2.5–3 hours.

In terms of limitations, in general based on our classroom experience, delivering lectures based on virtual labs is time-consuming, a lot of planning which involved many group discussions and one-to-one discussions with the PhD students involved. However, this positively contributes to the engagement of the classroom and for the PhD students this provided a great opportunity to transfer their knowledge to the undergraduate students. Learning through virtual experiments, followed by developing a chemical engineering problem in the classroom based on the data collected from virtual experiments, and subsequently solving that problem, despite being unconventional, directly conveys a clear message to students about how the real world looks and how it can be solved using chemical engineering theories—an aspect often missing in textbook problems. This approach is particularly well-suited for imparting practical knowledge about unit operations that may pose challenges in their implementation as part of traditional chemical engineering laboratory experiments.

For example, gravitational column chromatography, commonly used in pharmaceutical industries to separate structurally similar compounds, suffers from slow kinetics, involves low flow rate of mobile phase, and thus cannot be completed within 2–3 hours. Such a process can be easily implemented using virtual experiments. Another example is within the field of crystallisation is the experiments on crystal growth of organic or inorganic compounds. Typically, crystal growth process is time consuming and requires several hours to make a complete study on the crystal growth kinetics of a model compound in any solvent. Additionally, to perform experiments on crystal growth and column chromatography require skilled personnel and, possibly, an expert to make and operate the column. These processes involve solvent handling, powder handling, and we may need to operate the column for several hours. In such cases, virtual experiments become resourceful, allowing the study of a wide range of operating variables—an impossibility with experiments designed as part of regular chemical engineering laboratory exercises due to time limitations. These aspects limit the intention to implement such processes as part of a regular laboratory experiments, as the experiment time will exceed a typical duration of a laboratory session. In such cases, virtual experiments will become resourceful and

even it allows to study the effect of a wide range of operating variables which remains impossible with the experiments designed as part of the regular chemical engineering laboratory exercises due to the time limitations. Another advantage of delivering lectures via virtual experiments is that it not only develops the problem-solving skills, but it also directly enhances the student's ability to solve the real-world problems and can benefit from the theory which they learn during the lecture hours.

List of supplementary information provided to support students and instructors

- (1) Theory_Model problems.docx - includes derivations of nucleation theories and example problems relevant to crystallisation fundamentals. It also contains model problems explaining how to design nucleation experiments using solubility data, along with model example problems based on virtual experiments on nucleation performed with solutions of different initial supersaturation, and model exam-type problems.
- (2) Nulceation_exp_S_1_54.mp4: Edited experimental footage of the nucleation experiments performed at $S = 1.54$. With a playtime of less than 9 minutes, it can be easily deployed during the lecture hours or during the tutorials that typically run for 1 – 2 h. In the video, the vial numbers are clearly marked. Students should be asked to check the vial numbers and follow the instructions provided in the edited footage.
- (3) EAdata_nonlinear_regression.xlsx - is a self-explanatory Microsoft Excel spreadsheet that demonstrates how nonlinear regression analysis is performed to calculate the nucleation rate.
- (4) Classroom_activity.docx - a model document that can be provided to students in the classroom on the day we deployed the nucleation experiment in virtual mode. Students are required to complete the activity using this document in the classroom.

CRediT authorship contribution statement

Mayank Vashishtha: Conceptualization, Data curation, Investigation, Writing – original draft. **Vasanth Kumar Kannuchamy:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Shubhangi Kakkar:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft. **Mahmoud Ranjbar:** Formal analysis, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ece.2024.07.001](https://doi.org/10.1016/j.ece.2024.07.001).

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