



LUNCH & LEARN 2023

Crystal16: Nucleation Rate from Induction Time

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Technobis Crystallization Systems, The Netherlands

Technobis
CRYSTALLIZATION SYSTEMS

During this session

- Introduction to Technobis instruments
- About Crystal16
 - What is Crystal16
 - How it works .. Solubility measurement
 - Application
- Setting up an experiment to determine solubility & MSZW
- Nucleation
 - What is nucleation
 - Types of nucleation
 - Classical nucleation theory
- Experimental section
 - Sample preparation
 - Programming an experiment on Crystal16
 - Analysis the data using software
- Case - Study: Effect of solvent composition on solubility, MSZW and nucleation rate of ascorbic acid
- Conclusions
- Q&A / Discussion / Play with the instrument

Technobis Crystallisation Systems

- **Platforms for accelerating crystallisation research**
- Developed a number of unique and proprietary technologies
- Global services in 3 major markets: Pharma, Agro and Fine Chemicals
- Active also in Food and Personal Care, Inks, Coatings, Oil and Academia
- Portfolio contains 3 products for: **crystallisation**, **process optimization** and **formulation** related research



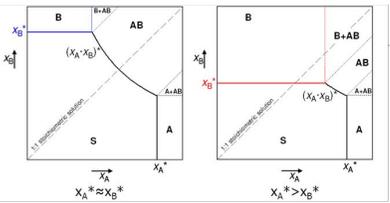
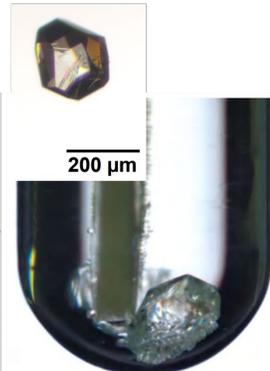
Products

Crystal BREEDER



Discovery

- ✓ Solubility, MSZW
- ✓ Polymorphs, Salt and Co-crystals screening
- ✓ Solvent screening
- ✓ Single crystal growth

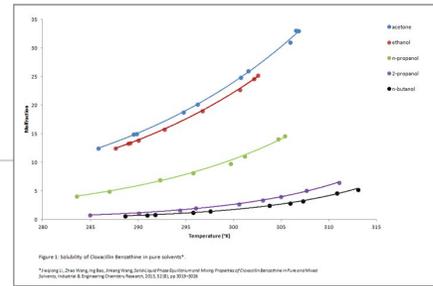
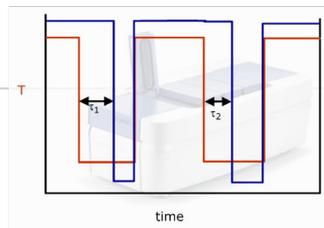


Crystal 16



Screening

- ✓ Solubility, MSZW
- ✓ Solvent screening
- ✓ Phase diagrams
- ✓ Nucleation analysis

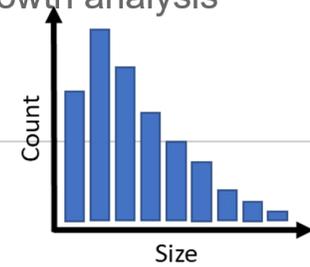


Crystal LINE



Optimization

- ✓ Habit control
- ✓ Particle size distribution
- ✓ Process optimization
- ✓ Formulation
- ✓ Growth analysis



Products

Discovery



Working volume:
0.06 – 0.1 ml
32 reactors



Screening



Working volume:
0.5 – 1.0 ml
16 reactors



Optimization



Working volume:
2.5 – 5 ml
8 reactors



Small amount of
material

In-line Analytics

No Cleaning required

Cheap disposal of the
reactors

Little training required



Crystal16: what is it...

Features

Crystal 16

Desktop parallel crystallizer for medium-throughput crystallization studies



16 reactors



Standard 1 ml disposable reactor vessels (HPLC vials)



4 independent temperature blocks



Temperature range from -20 °C to 150 °C (-25 °C with chiller)



Magnetic/overhead stirring in each reactor



Turbidity measurement in each reactor



Intuitive software



A critical workflow component in your high-end research and pre-production lab environment

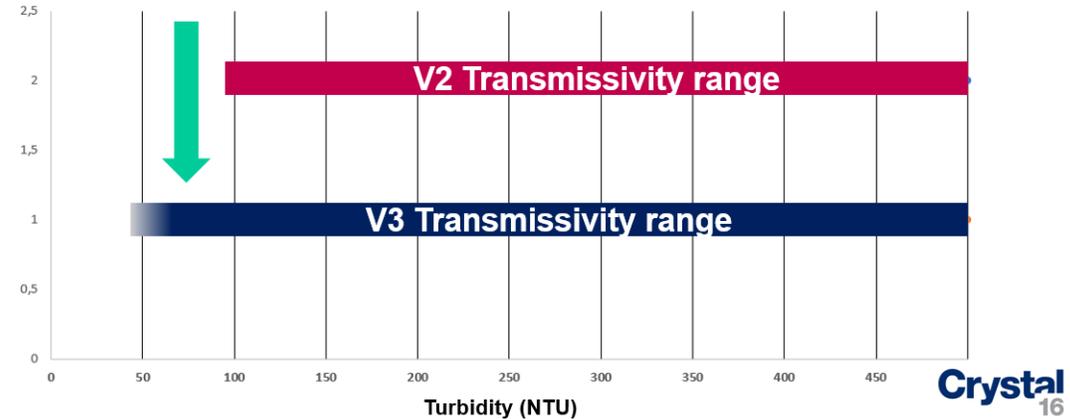
End-User Benefits of the Crystal16V3

INCREASED LIMITS OF DETECTION AND IMPROVED TRANSMISSIVITY SENSOR LINEARITY

Better results for both low and highly concentrated samples

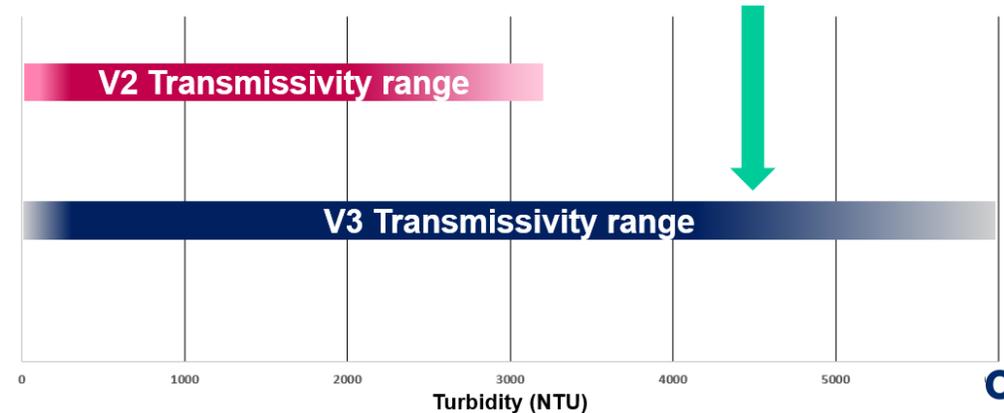
LOWER LIMIT OF DETECTION

MEASURE LOWER CONCENTRATIONS



HIGH LIMIT OF DETECTION

MEASURE MORE OPTIQUELY OPAQUE SAMPLES



✓ Improved Experimental Results

Improved Data Processing

Ruggedness & Reliability

End-User Benefits of the New Crystal16

Improved Experimental Results

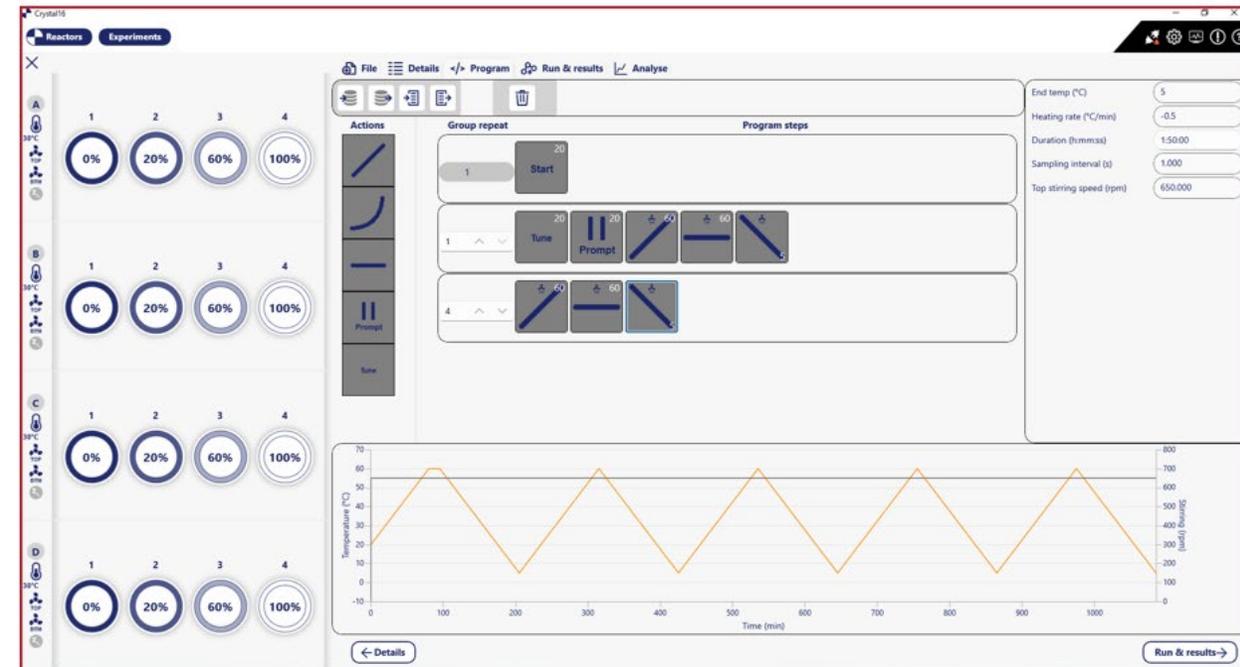
NEW INTUITIVE SOFTWARE

- Redesigned intuitive block-based experiment programming interface
- User friendly, easy to use, flexible

EASY INTUITIVE EXPERIMENT PROGRAMMING USING LOGIC BLOCKS

✓ Improved Data Processing

Ruggedness & Reliability



End-User Benefits of the New Crystal16

Improved Experimental Results

INTEGRATED SOFTWARE FOR DATA ANALYSIS

- Integrated data collection and processing
- User defined clear and cloud points
- Online analysis of results

✓ Improved Data Processing

Ruggedness & Reliability

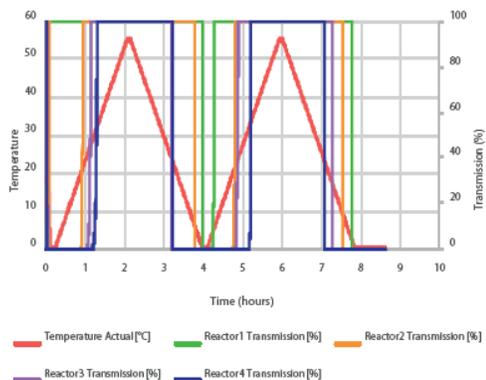
CrystallizationSystems
Experiment Programming
and Monitoring

CrystallizationSystems2
Integrated Software

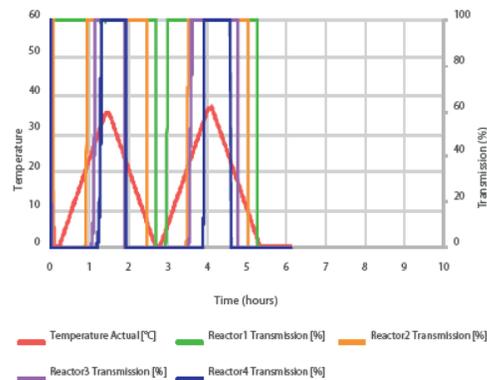
CrystalClear
Data Analysis

New Feature: Feedback control

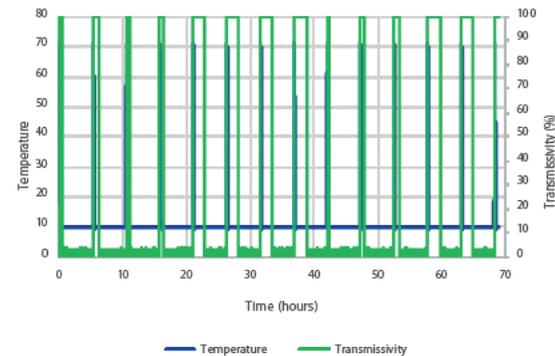
- Feedback control allows for the reduction of experimental time
- End heating ramp once a sample reaches 100 % transmissivity (solubility point)



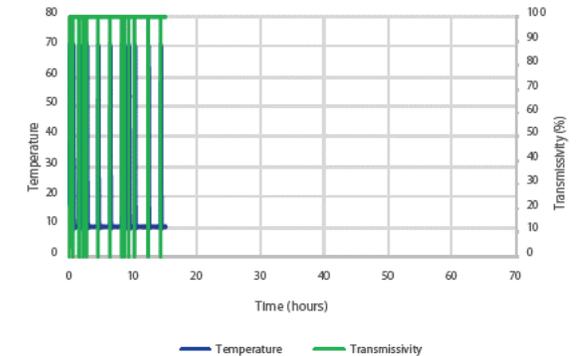
Solubility *without* feedback control



Solubility *with* feedback control



Crystallization *without* feedback control



Crystallization *with* feedback control

Benchmark Crystal16 V2 vs V3

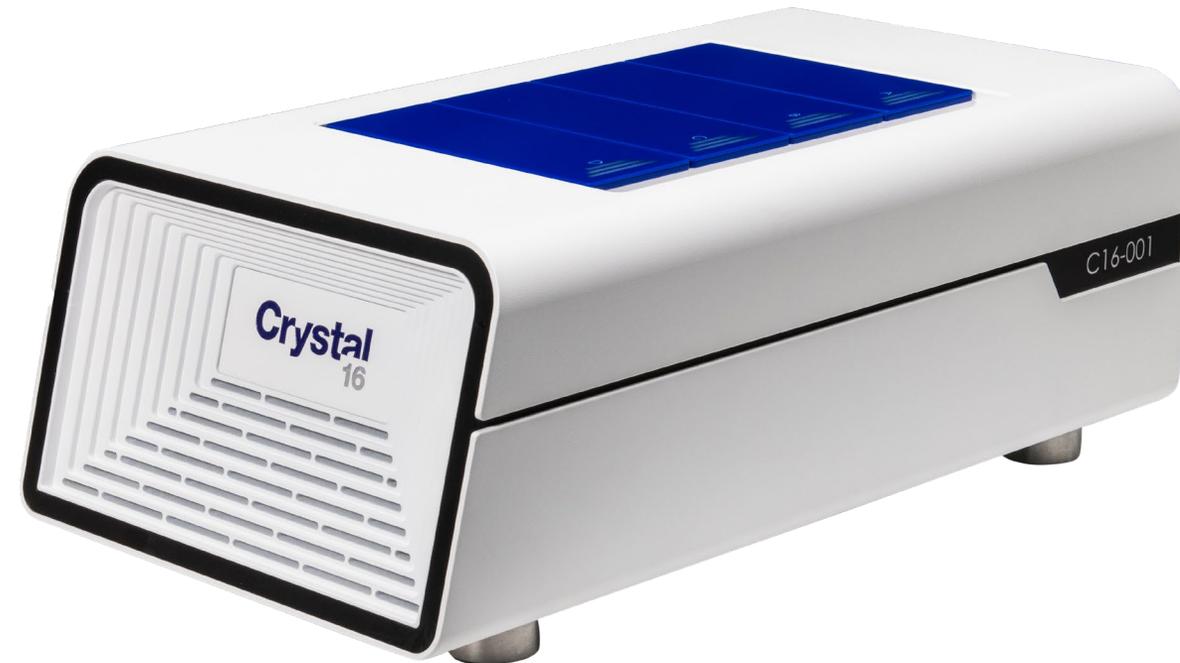


Aspect	Crystal16 V2	Crystal16 V3
Temperature range	-15C on 1 reactor blocks -10C on all 4 reactor blocks	-20 on ALL 4 reactor blocks (Air cooled) -25C on ALL 4 reactor blocks (external chiller)
Transmissivity	(*system dependent; example on CBZ in IPA/water, min amount 1 mg/mL)	Linear over a wider range; Improved response for more optically opaque samples; Improved low level sensitivity: detect 50% less material* (*system dependent; example on CBZ in IPA/water, min amount 0.5 mg/mL)
Maintenance and serviceability	Constructed from several parts	Unibody – quicker maintenance, less downtime Less purge gas consumption
Data Analysis	Separate software for data acquisition and for data analysis Fixed automated clear and cloud point analysis	Integrated data acquisition and analysis in a single software User defined automated clear and cloud point analysis Feedback control

Solubility

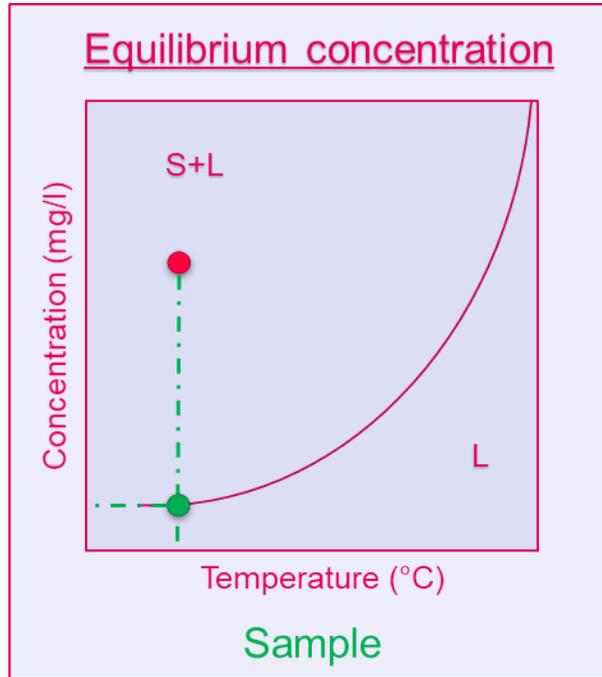
Why and when solubility matters?

- To determine the Solubility curve and MSZW
- Solvent selection for crystallization
- Design crystallization process
- To determine theoretical yield
- Counter-ion selection for salt formation
- Co-former selection for co-crystallization
- Impurity impact – purification/separation method
- Impact of different temperature profiles on your crystallization process



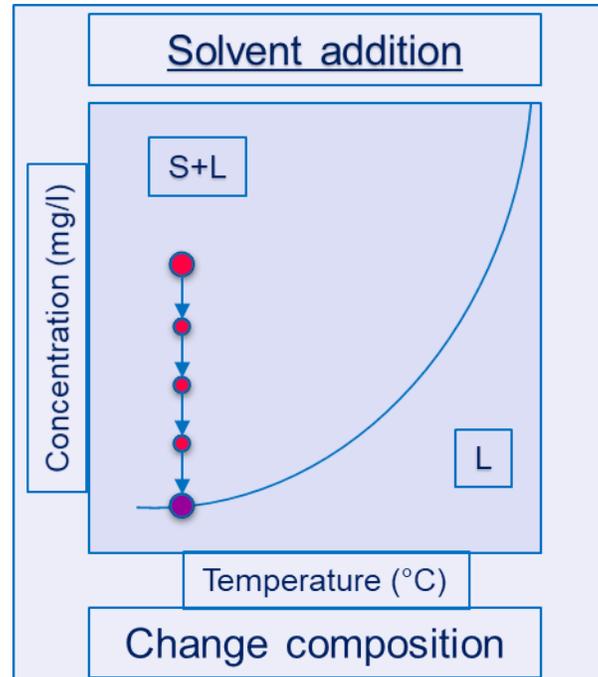
How to determine the solubility

Gravimetric method



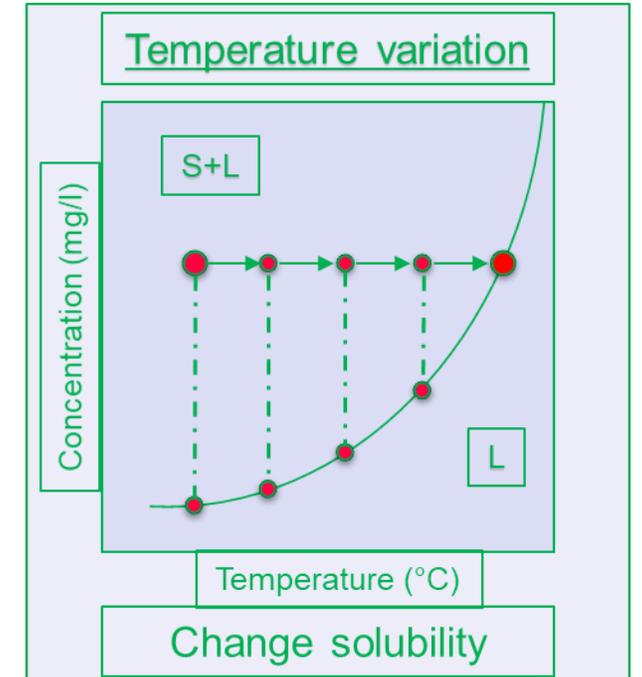
4 samples = 2 Hrs sample prep + 8 hrs ground work (calibration curve)
72 hrs equilibrations
Most accurate

Solvent Additional method



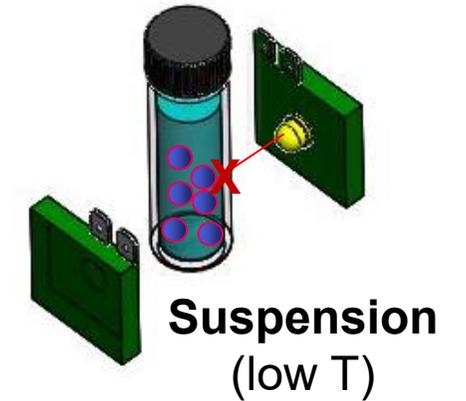
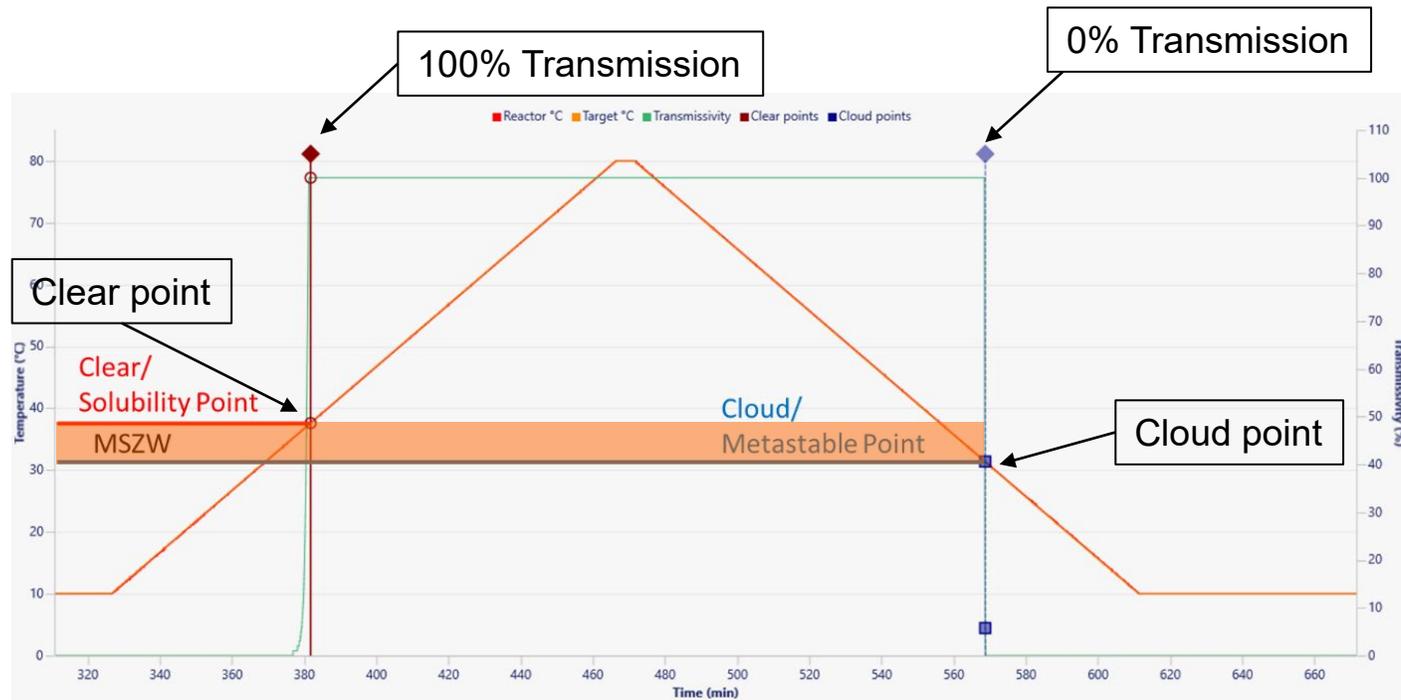
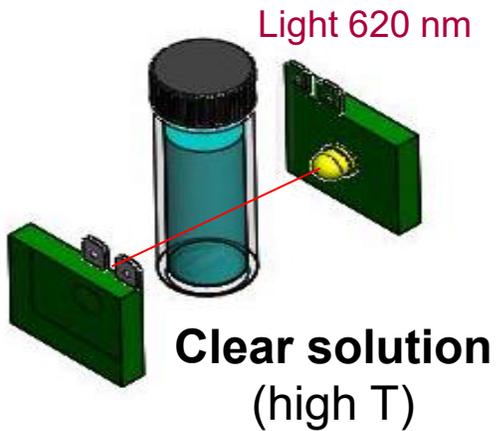
4 samples = 6 Hrs

Polythermal method (C16)

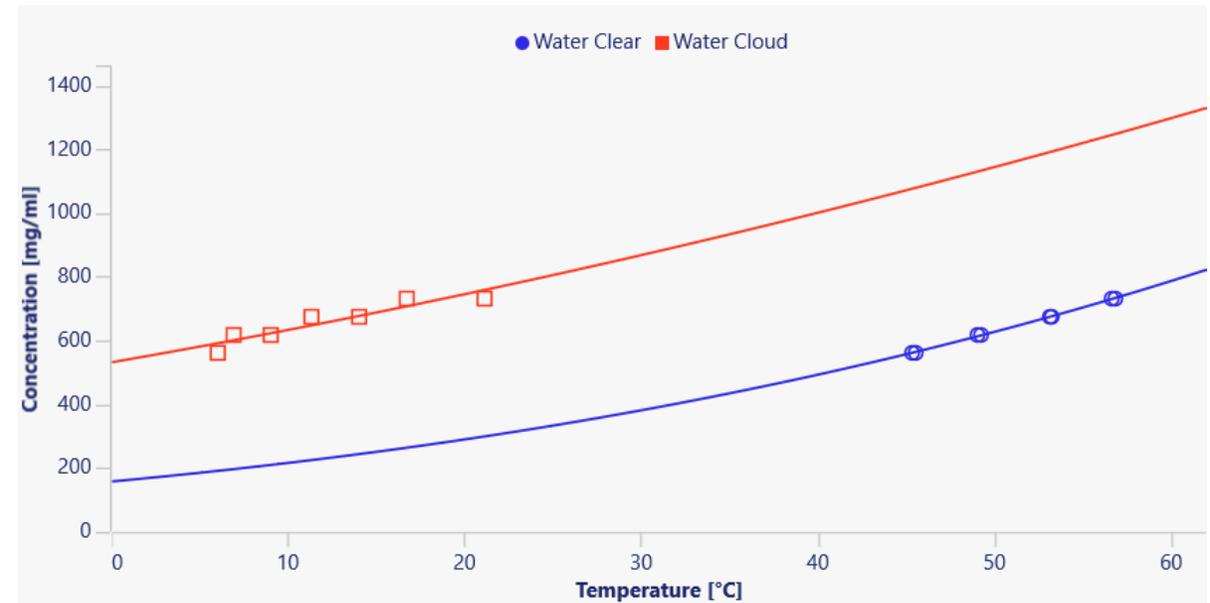
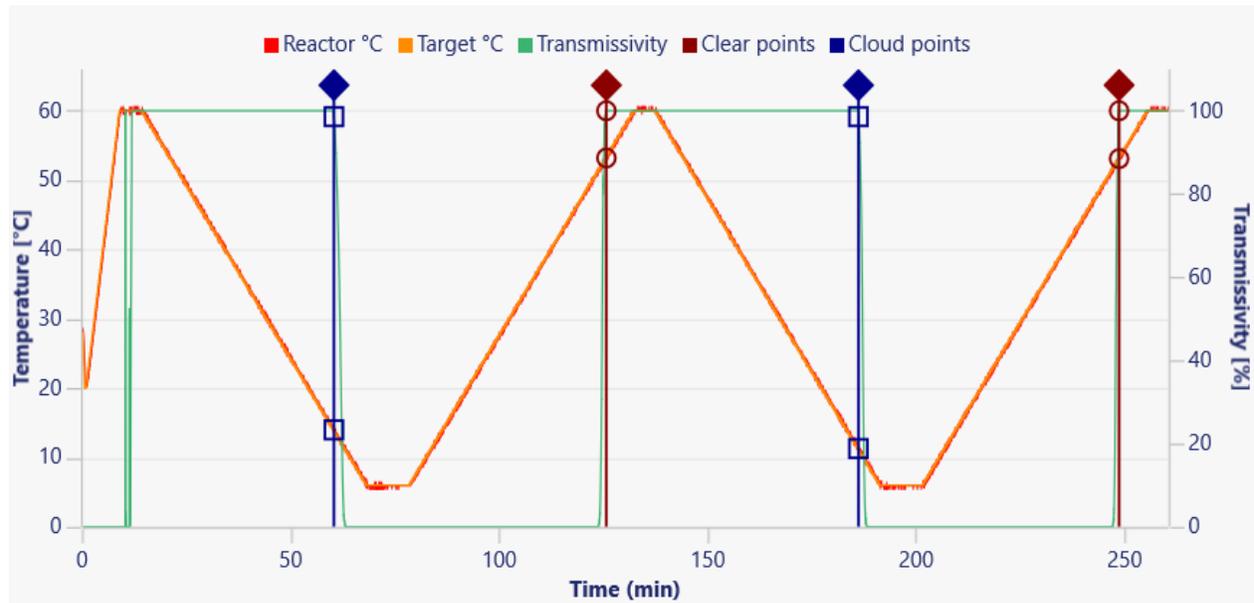


16 Samples = 2 Hrs sample Measurement automated overnight
2 work hours total

How it works: Transmissivity Technology

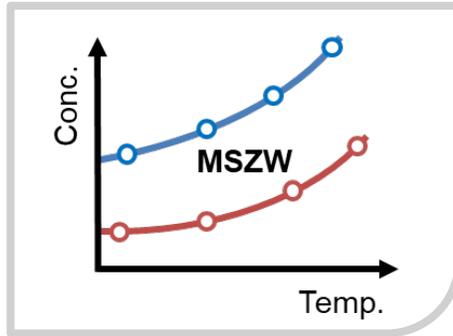


Solubility Curve and MSZW

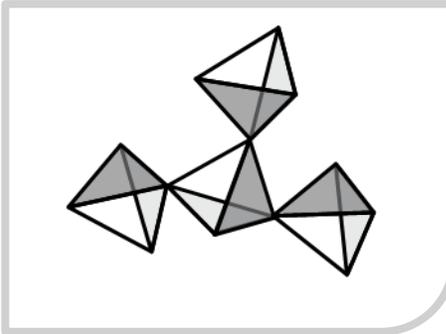


Crystal16 v3 – Ready For Almost Any Crystallization Application

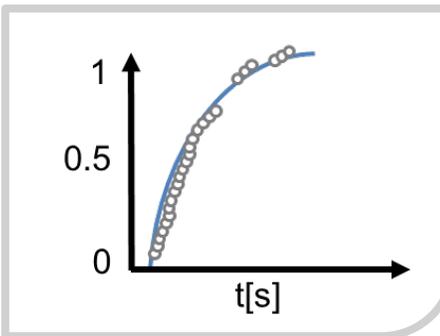
Solubility & MSZW



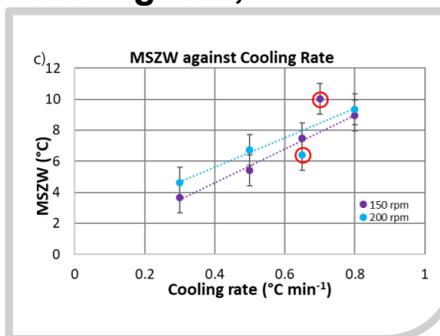
Solid State Screening



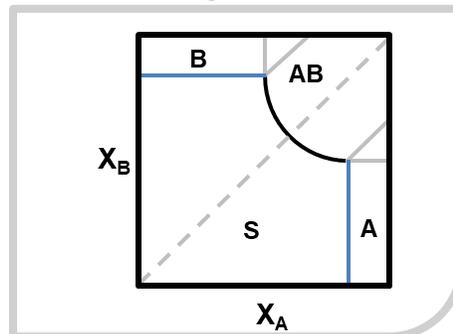
Induction Times & Nucleation Rates



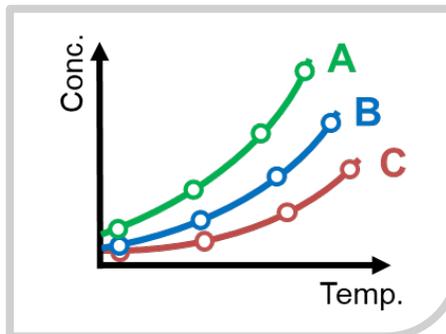
Experiment conditions: cooling rate, RPM



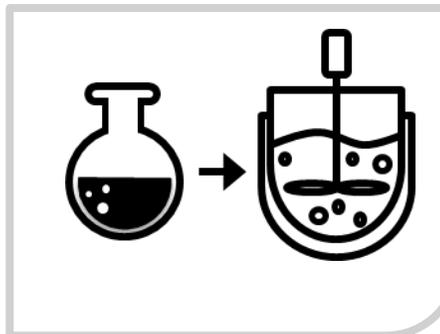
Phase Diagrams



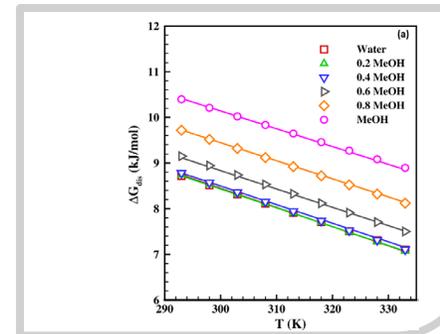
Solvent Screening



Crystallization Scale-Up



Thermodynamic studies



Solubility & MSZW determination experiment setup

Ascorbic acid in Water:

Details: Concentrations

Solubility of Ascorbic acid in Water

Vials	Vial 1	Vial 2	Vial 3	Vial 4
Compound	Ascorbic acid	Ascorbic acid	Ascorbic acid	Ascorbic acid
Solvent	Water	Water	Water	Water
Concentration	561,0000	617,0000	674,0000	731,0000
Supersaturation	0,00	0,00	0,00	0,00
Unit	mg/ml	mg/ml	mg/ml	mg/ml
Top stirrer				
Bottom stirrer	Standard	Standard	Standard	Standard
	Clear Copy to all			

Program

File Details **Program** Run & results Analyse

End temperature (°C) 60
Duration (h:mm:ss) 0:05:00
Sampling interval (s) 1
Bottom stirring speed (rpm) 800

Group repeat	Program steps
1	START 20°
0/1	60° 60° TUNE 60°
0/2	6° 6° 60° 60°

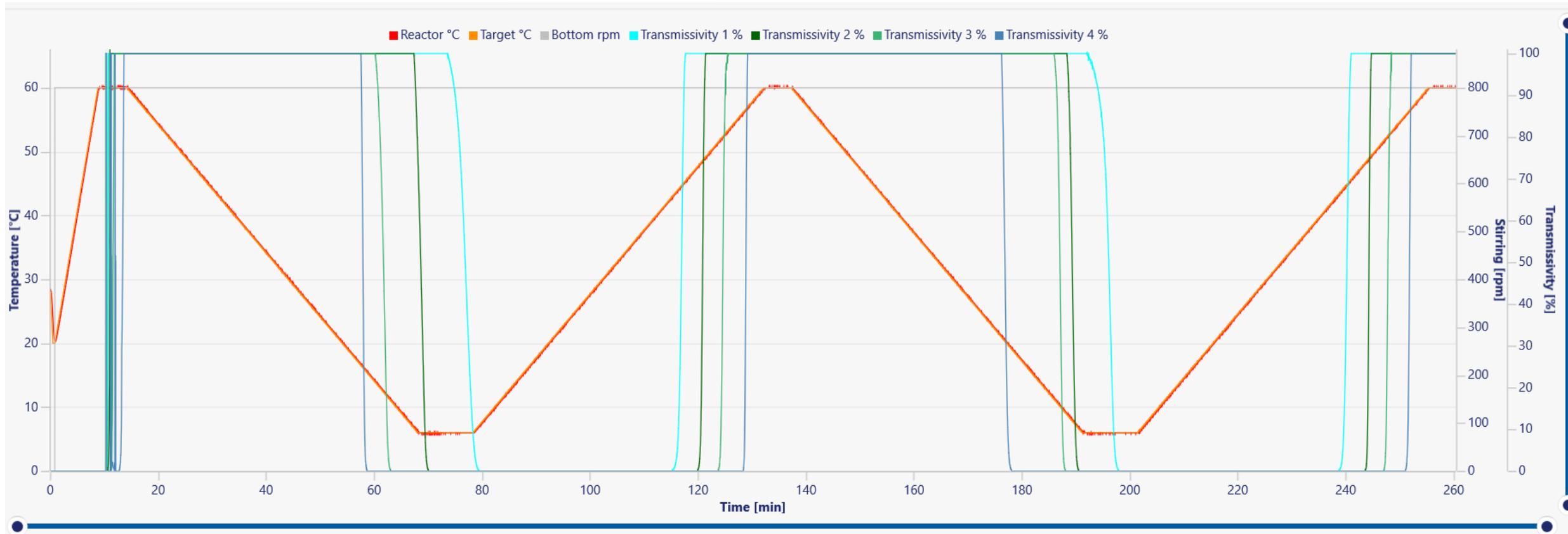
Temperature [°C] 66 60 40 20 0

Time [min] 0 20 40 60 80 100 120 140 160 180 200 220 240 259,016666666667

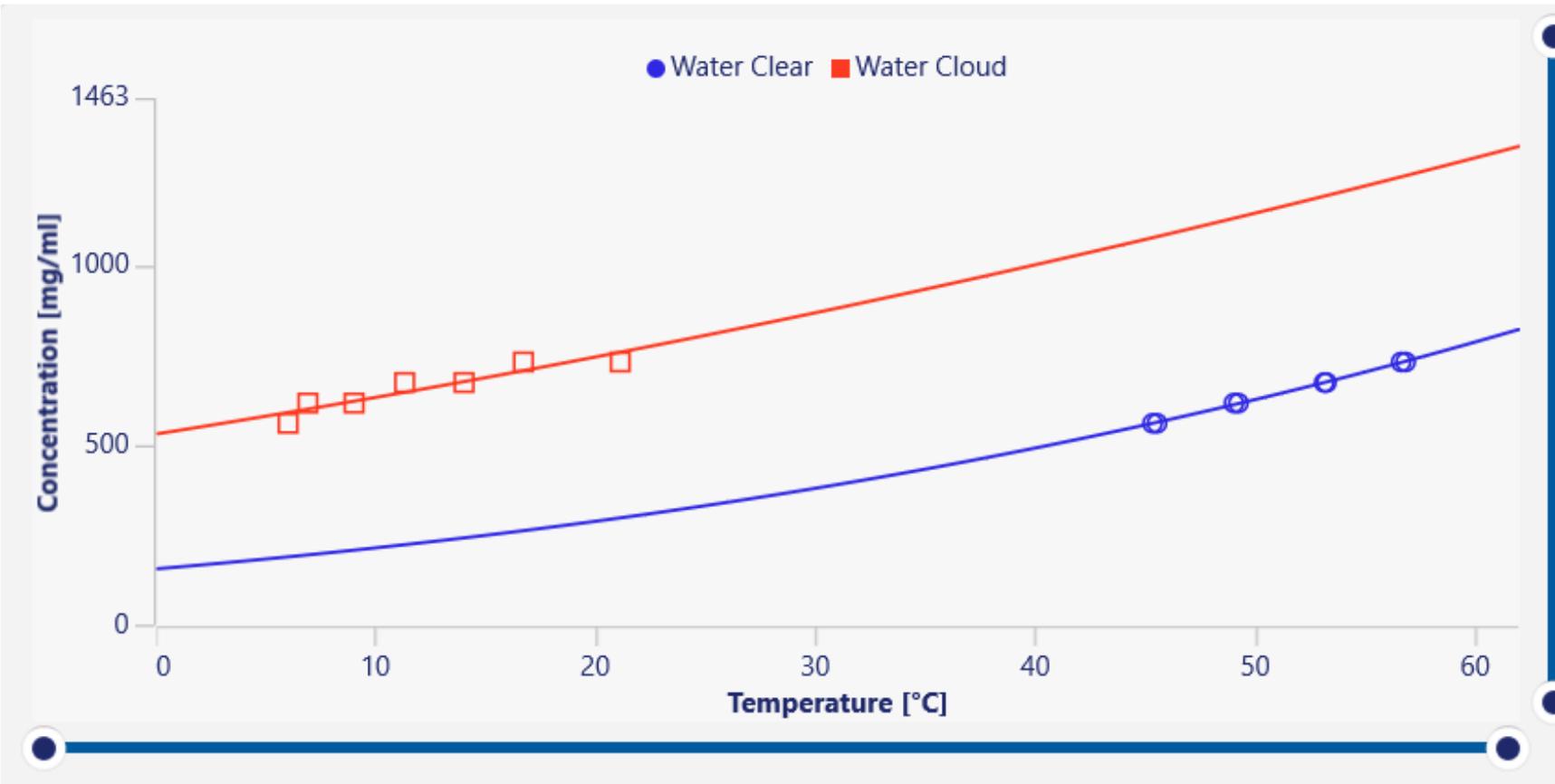
Stirring [rpm] 880 800 600 400 200 0

Reactor °C Bottom rpm

Transition points

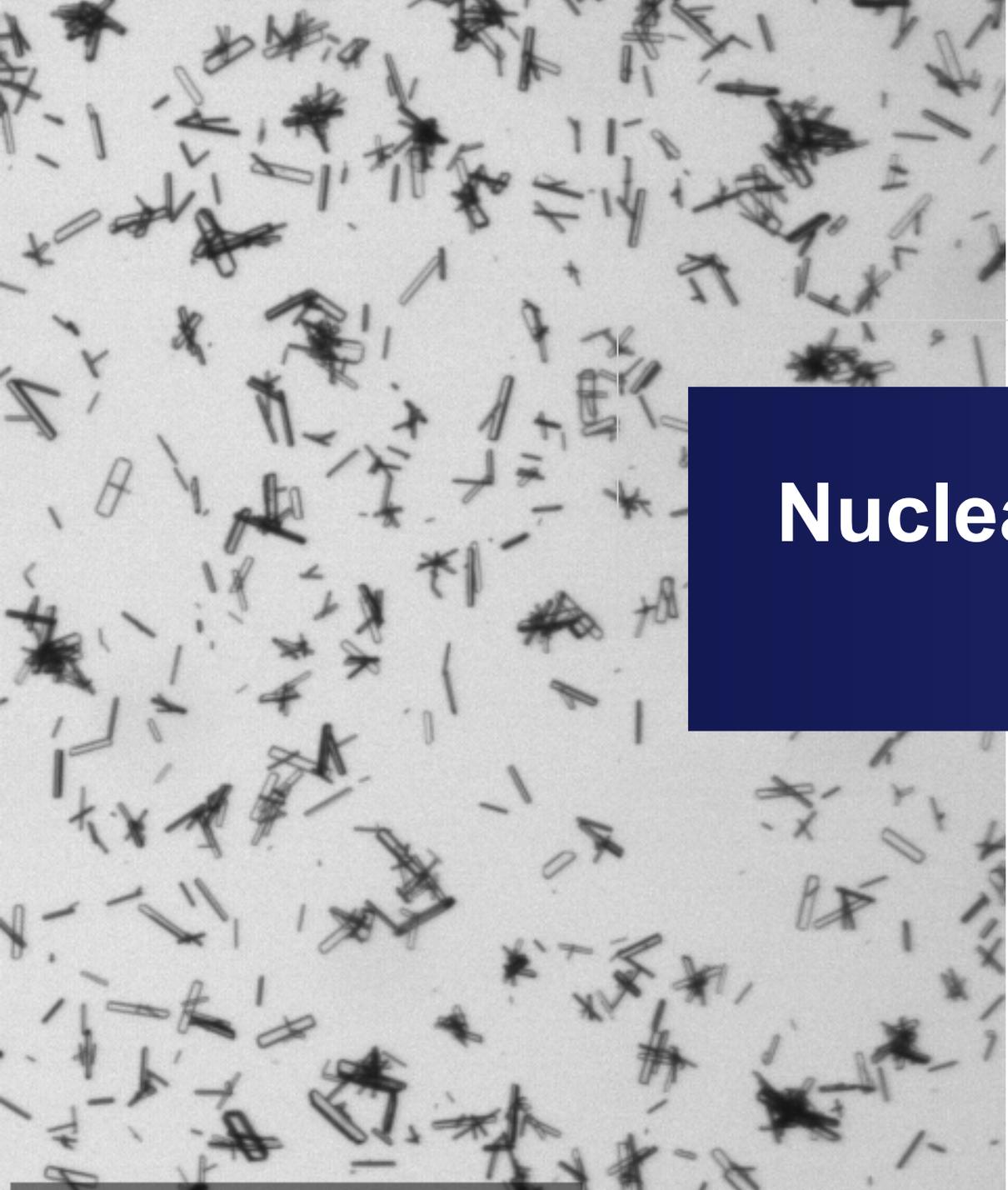


Solubility & MSZW



Solubility curves

Solvent	Point type	Function	Color	R2	AdjR2	Fitted function
Water	Clear	Van't Hoff		0.9989	0.999	$\exp(13,9946 - 2439,6478 / (T + 273))$

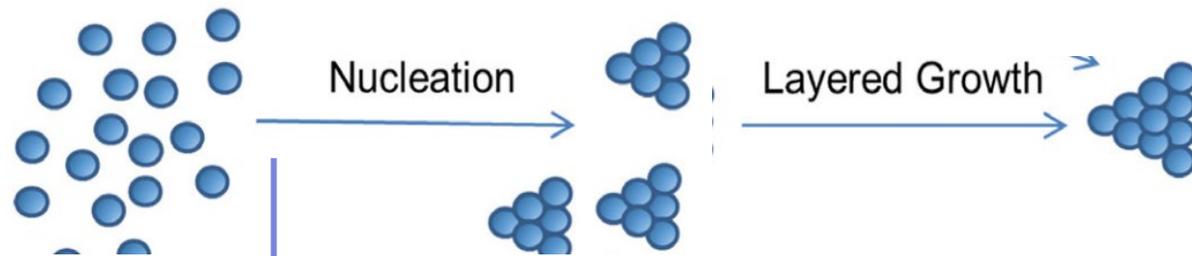


Nucleation

- What is nucleation?
- Methods to measure nucleation
- Induction time measurement method

What is nucleation?

Nucleation is the process of creation of a solid phase from liquid phase

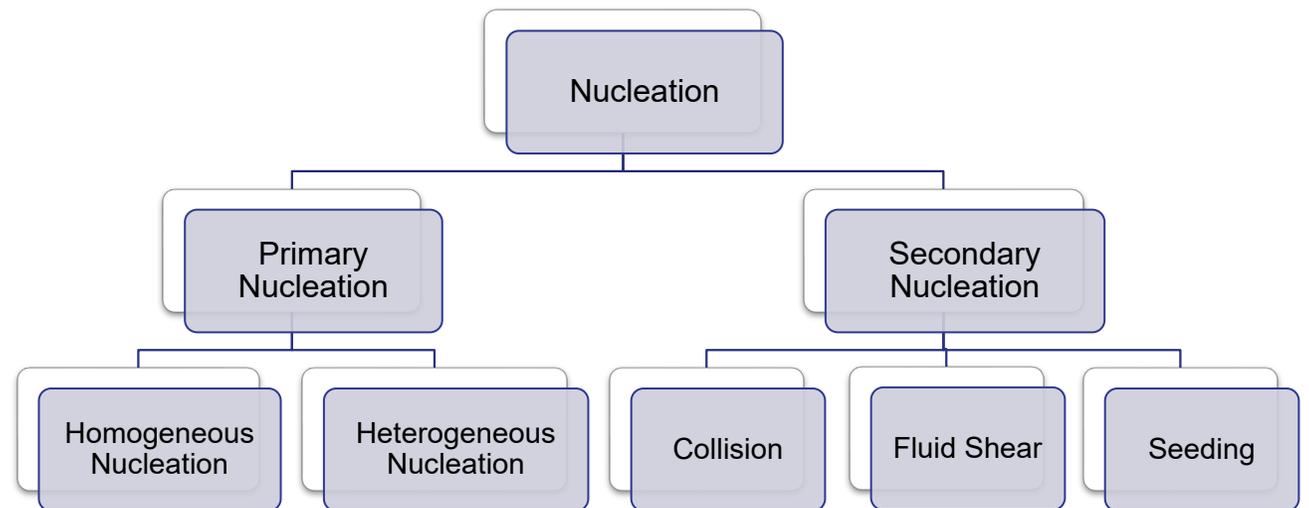


Polymorph

Crystal size

Crystal shape

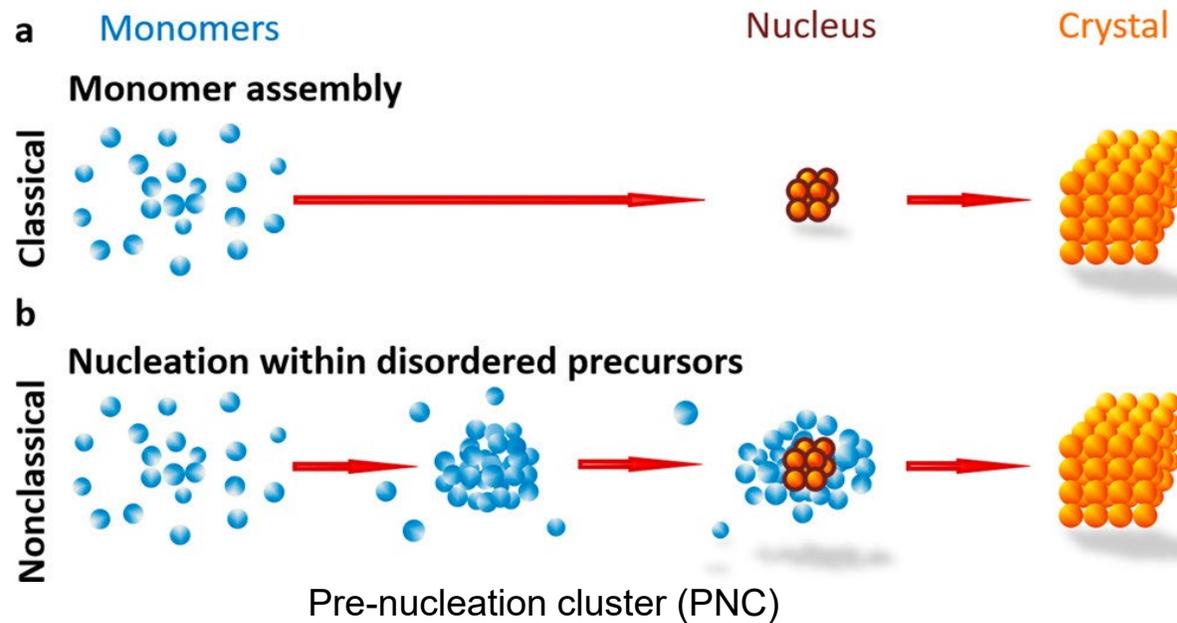
Particle size distribution



Nucleation Theory

The mechanism for nucleation is unknown, but two mechanisms are proposed

- Classical nucleation theory
- Non-Classical Nucleation theory



Classical Nucleation Theory (CNT)

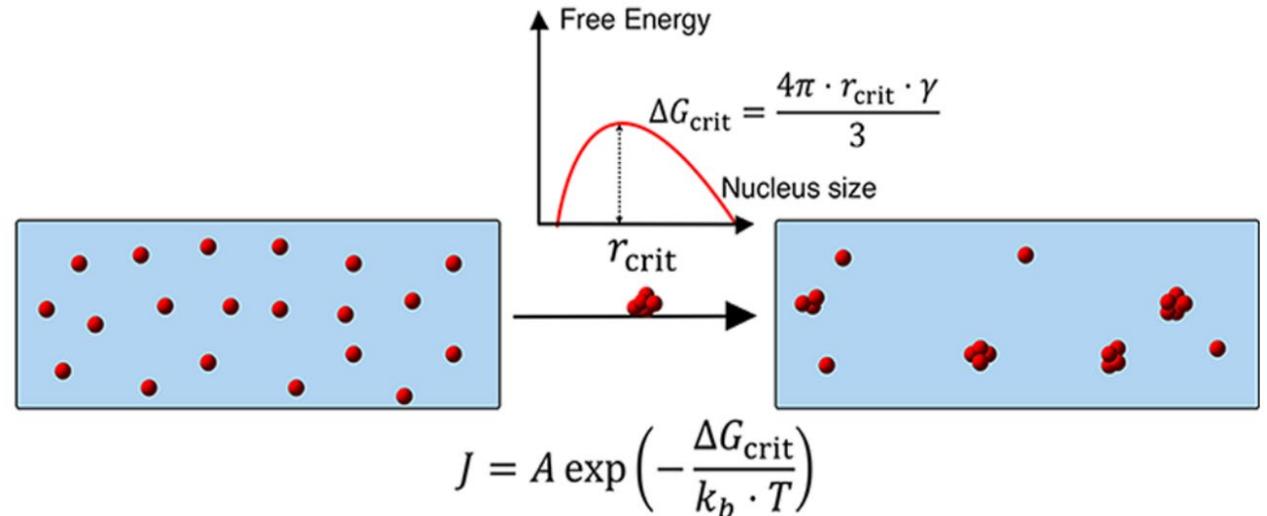
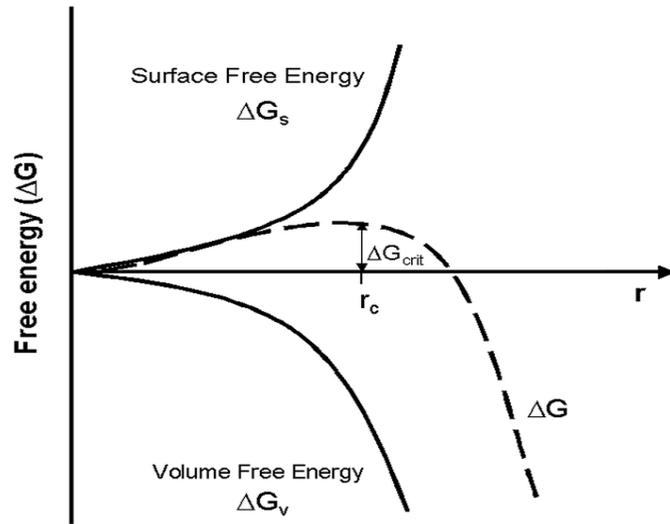


Fig: Schematic showing the dependence of nucleation barrier ΔG on the radius r according to classical nucleation theory.

$$J = A S \exp\left(\frac{-B}{\ln^2 S}\right)$$

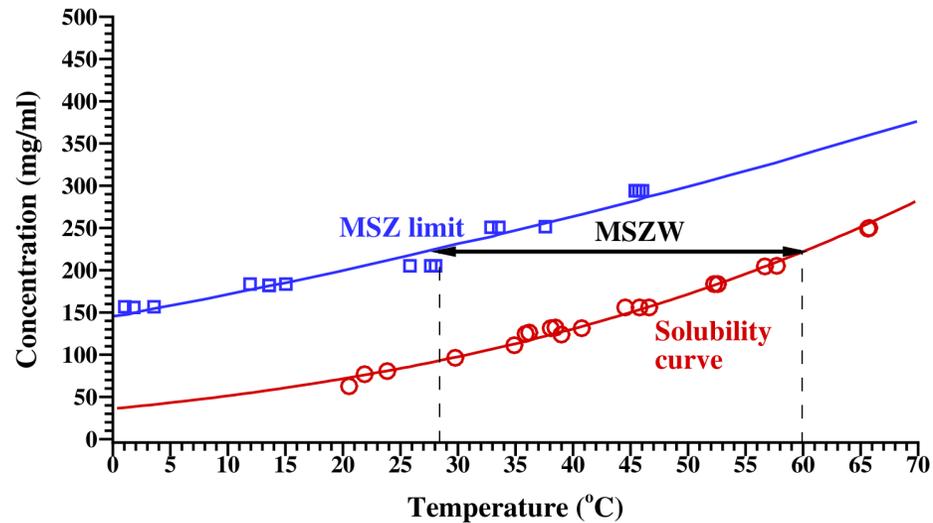
$$A = \frac{f^* C_0 z}{S}$$

$$B = \frac{16\pi\gamma^3\vartheta^2}{3k_b^3 T^3}$$

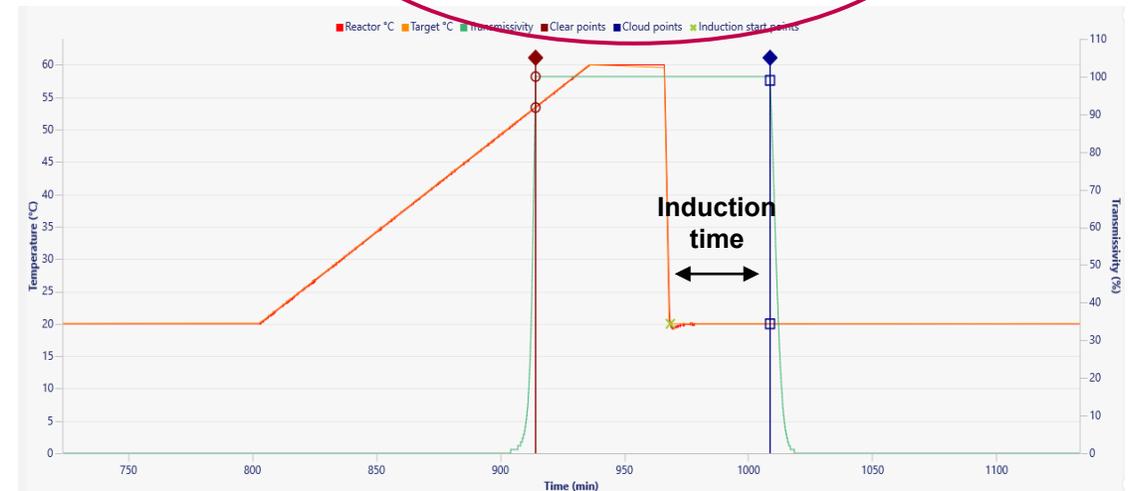
- f^* = attachment frequency
- C_0 = concentration of active nucleation site
- Z = Zeldovich factor
- γ = interfacial tension
- ϑ = molecular volume
- k_b = Boltzman constant
- T = Temperature
- S = supersaturation

Methods to measure nucleation kinetics

Polythermal method Metastable zone width & Cooling rate



Isothermal method Induction time & Supersaturation ratio



Theory

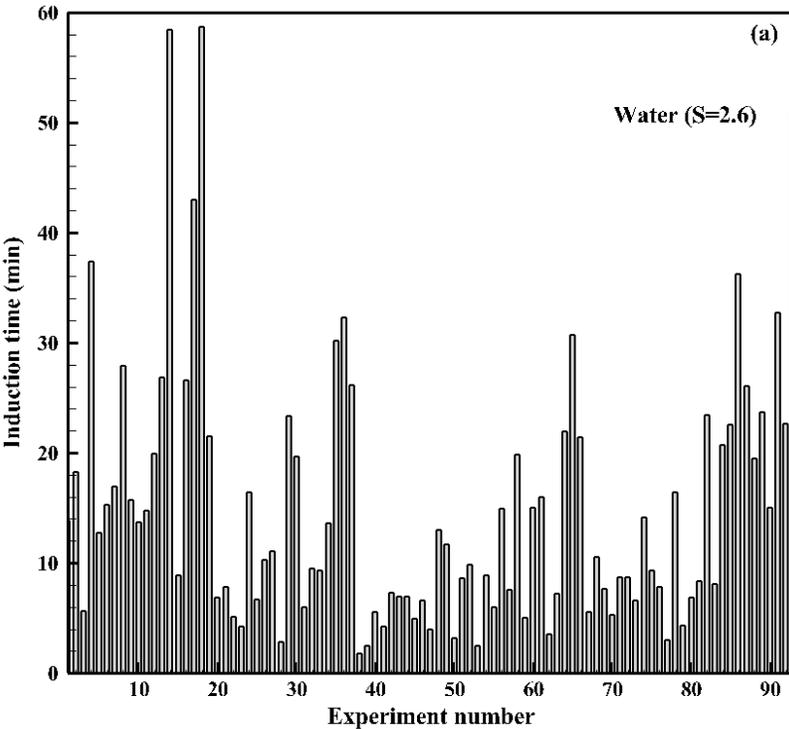


Fig: Induction time over 93 experiments.

N : no of nuclei

J : nucleation rate

S : supersaturation

A : kinetic factor

B : thermodynamic factor

M : total number of measurements

$M(t)$: number of measurements in the timeframe t .

The probability of the formation of a nucleus in a certain timeframe can be described by Poisson distribution:

$$P_m = \frac{N^m}{m!} \exp(-N)$$

$$N(t) = JVt_j$$

The probability $P(t)$ to form one or more nuclei is:

$$P(t) = 1 - P_0 = 1 - \exp(-JVt_j)$$

$$t_j = t - t_g$$

$$P(t) = 1 - \exp(-JV(t - t_g))$$

$$P(t) = \frac{M(t)}{M}$$

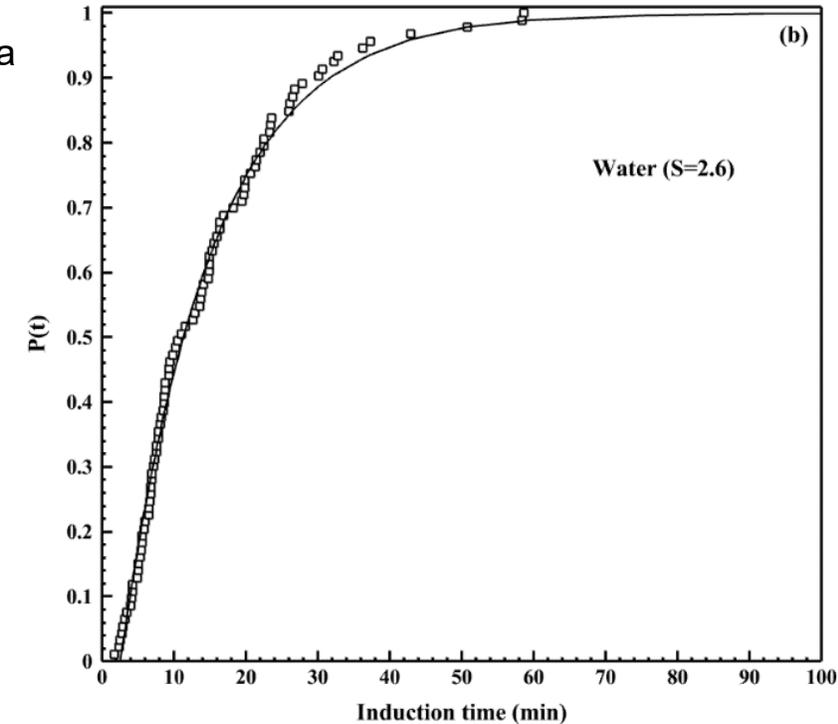
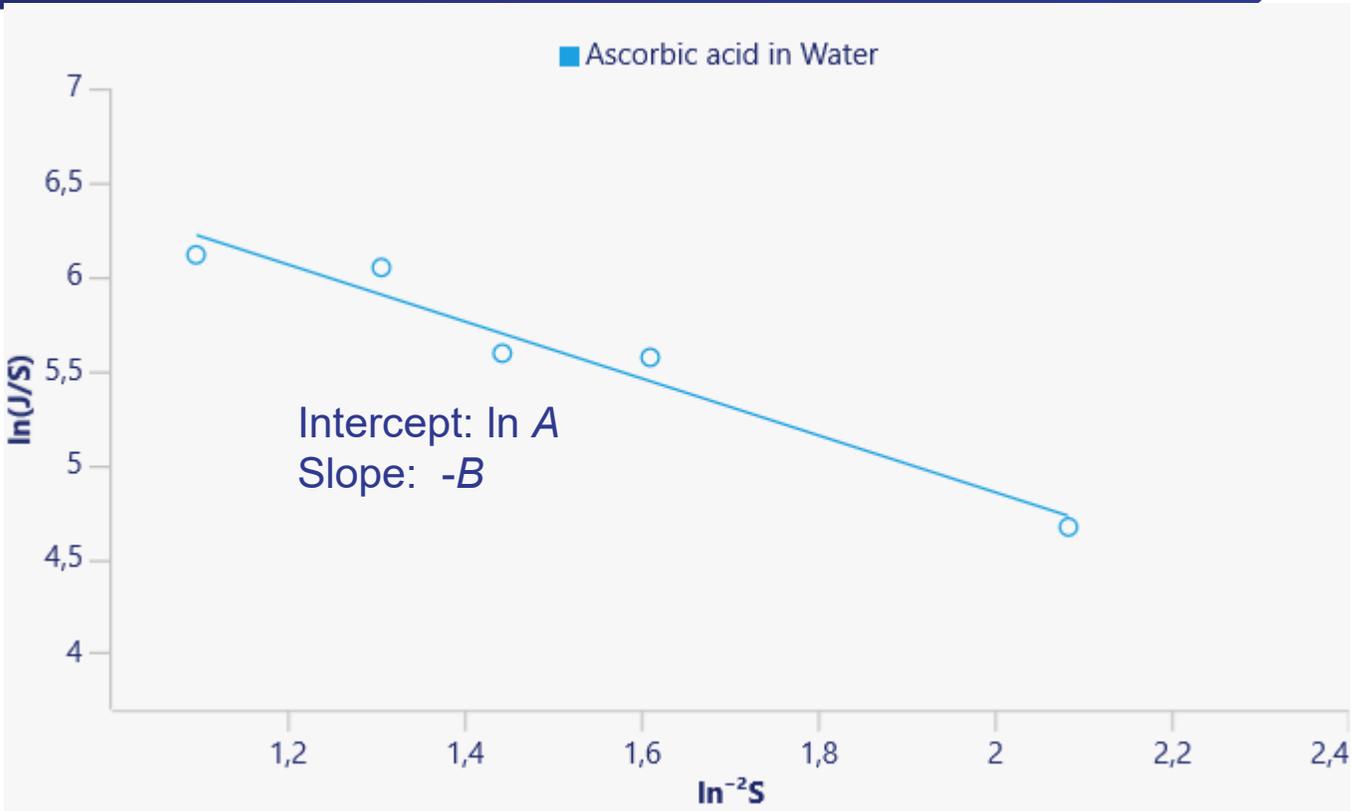


Fig: Probability distribution vs induction time.

Theory

Supersaturation	Fitted function	J [m ⁻³ s ⁻¹]	tg [s]
2.4	$P(t) = 1 - \exp(-1015,84 * 1e-6 * (t - 555,06))$	1015.84	555.057
2.2	$P(t) = 1 - \exp(-577,38 * 1e-6 * (t - 721,43))$	577.38	721.433
2.6	$P(t) = 1 - \exp(-1177,13 * 1e-6 * (t - 158,11))$	1177.13	158.112
2	$P(t) = 1 - \exp(-213,12 * 1e-6 * (t - 2234,98))$	213.124	2234.98
2.3	$P(t) = 1 - \exp(-617,01 * 1e-6 * (t - 409,17))$	617.005	409.169

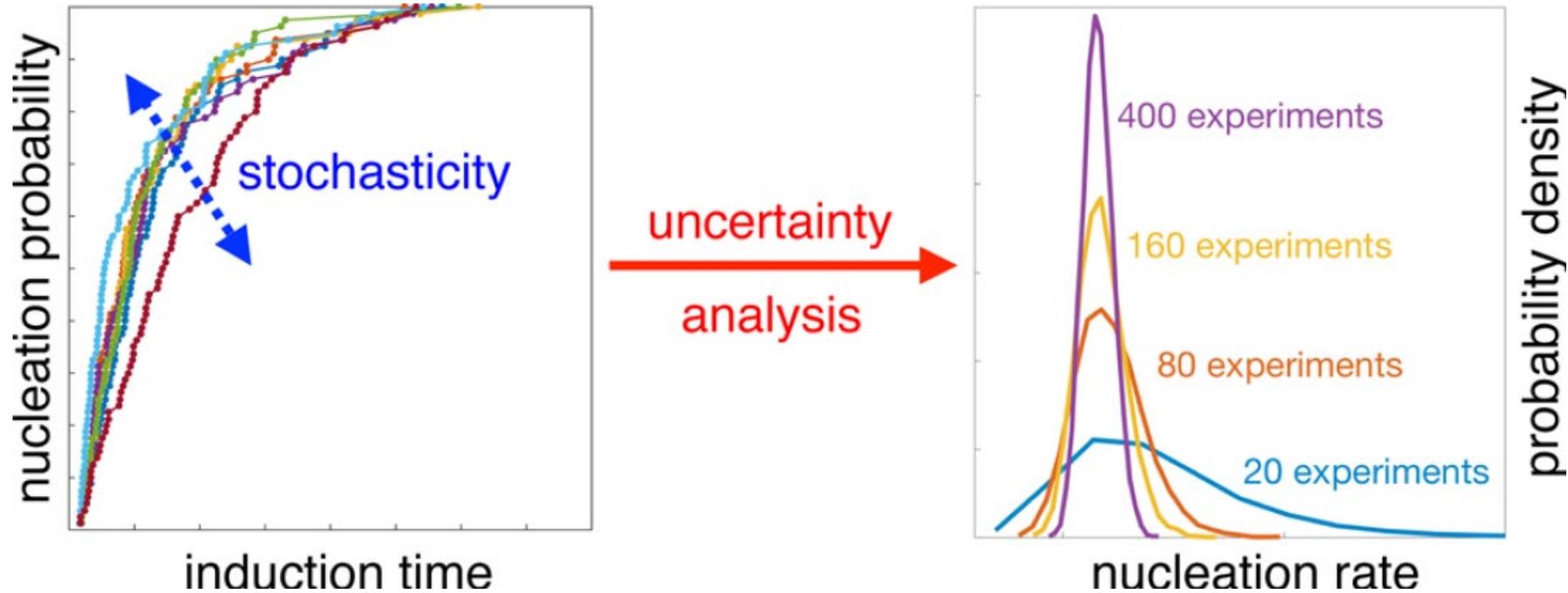
$$\ln\left(\frac{J}{S}\right) = \ln A - \frac{B}{\ln^2 S}$$



Nucleation rates table

Compound	Solvent	Color	Fitted function]	A [m ⁻³ s ⁻¹]	B	R2	Adj R2
Ascorbic acid	Water		$J(S) = 2642,314 * S * \exp(-1,5141 / \ln^2(S))$	2642.31	1.51405	0.9533	0.9065

Number of experiments

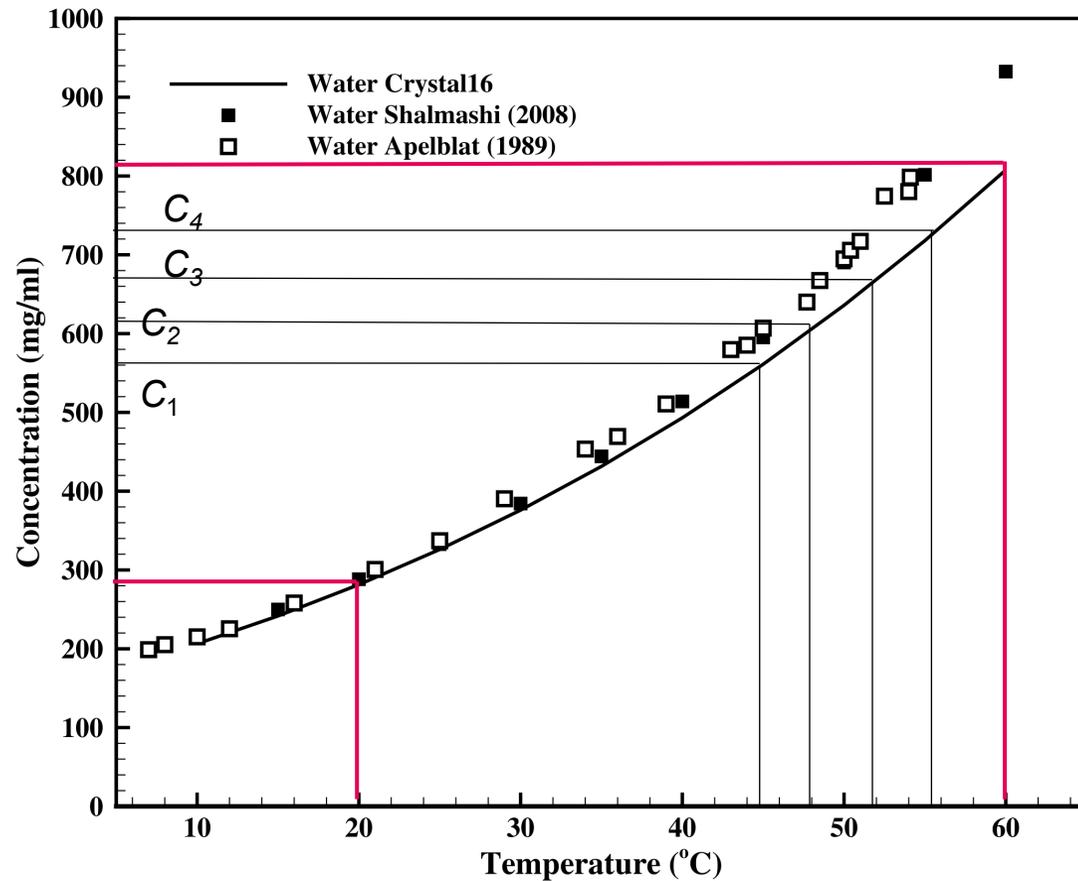


“Under these circumstances, our data modeling showed that 80 or more induction times should be measured in the real experiments, that setting growth time, t_g , as the shortest induction time is acceptable and requires no additional information about the growth rate, and that using a nonlinear fitting method to the Poisson equation is the best option for estimating the nucleation rate.”



Experimental Section

Preparing samples



$$S = \frac{C}{C^*}$$

- $C_1 = 561 \text{ mg/ml}$
- $C_2 = 617 \text{ mg/ml}$
- $C_3 = 674 \text{ mg/ml}$
- $C_4 = 731 \text{ mg/ml}$

4 supersaturations

$S_1 = 2.0$ $S_2 = 2.2$ $S_3 = 2.4$ $S_4 = 2.6$



HPLC vials

- S = supersaturation ratio
- C = concentration (mg/ml)
- C^* = equilibrium concentration (mg/ml)

Setting up experiment



Crystal16 V3

File Details Program Run & results Analyse

Experiment name: Ascorbic acid in water Description: Optional

Experiment type: FeedbackControl

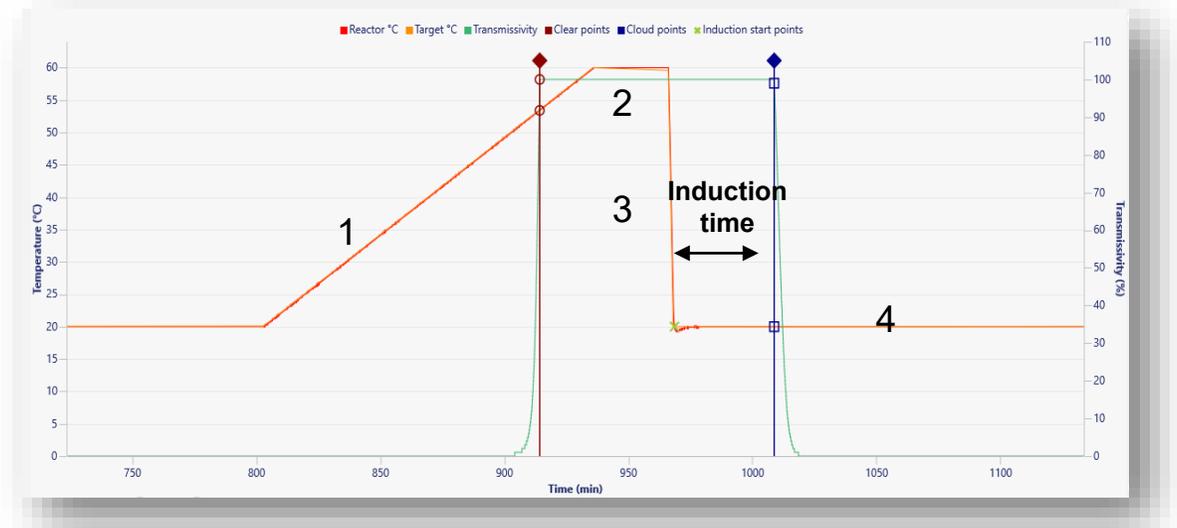
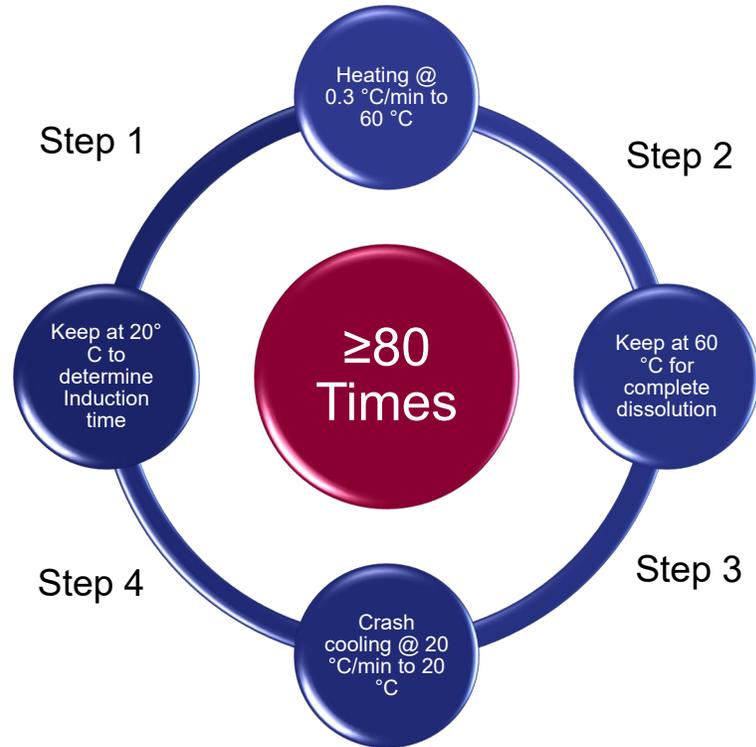
User name: Optional

Cap type: Basic cap

Target reached after: 3 Seconds Trigger on vial: All

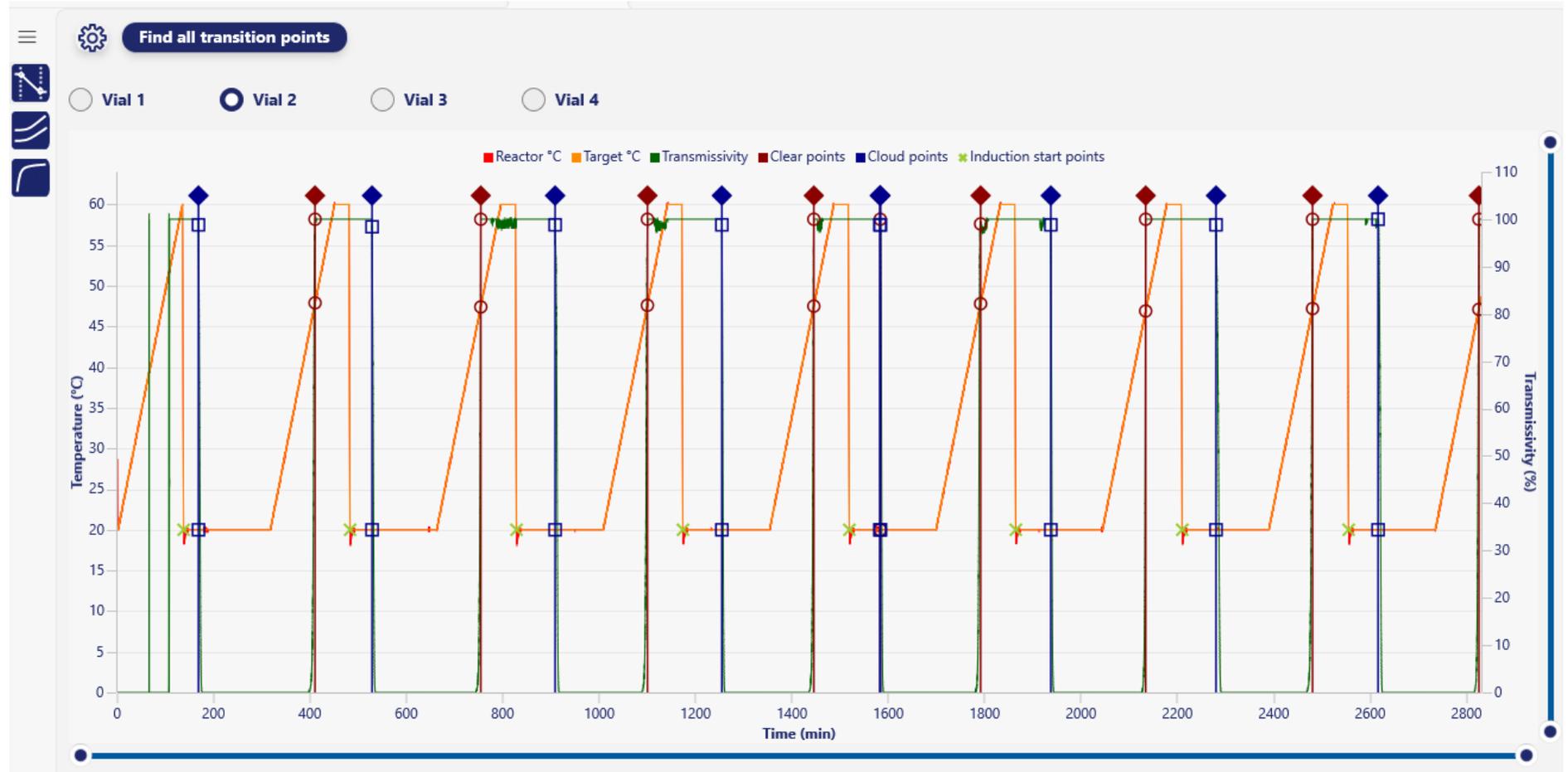
Vials	Vial 1	Vial 2	Vial 3	Vial 4
Compound	Ascorbic acid	Ascorbic acid	Ascorbic acid	Ascorbic acid
Solvent	Water	Water	Water	Water
Concentration	561.8000	617.3000	674.8000	730.8000
Supersaturation	2.00	2.20	2.40	2.60
Unit	mg/ml	mg/ml	mg/ml	mg/ml
Top stirrer				
Bottom stirrer	Standard	Standard	Standard	Standard
	Clear Copy to all			

Experimental procedure

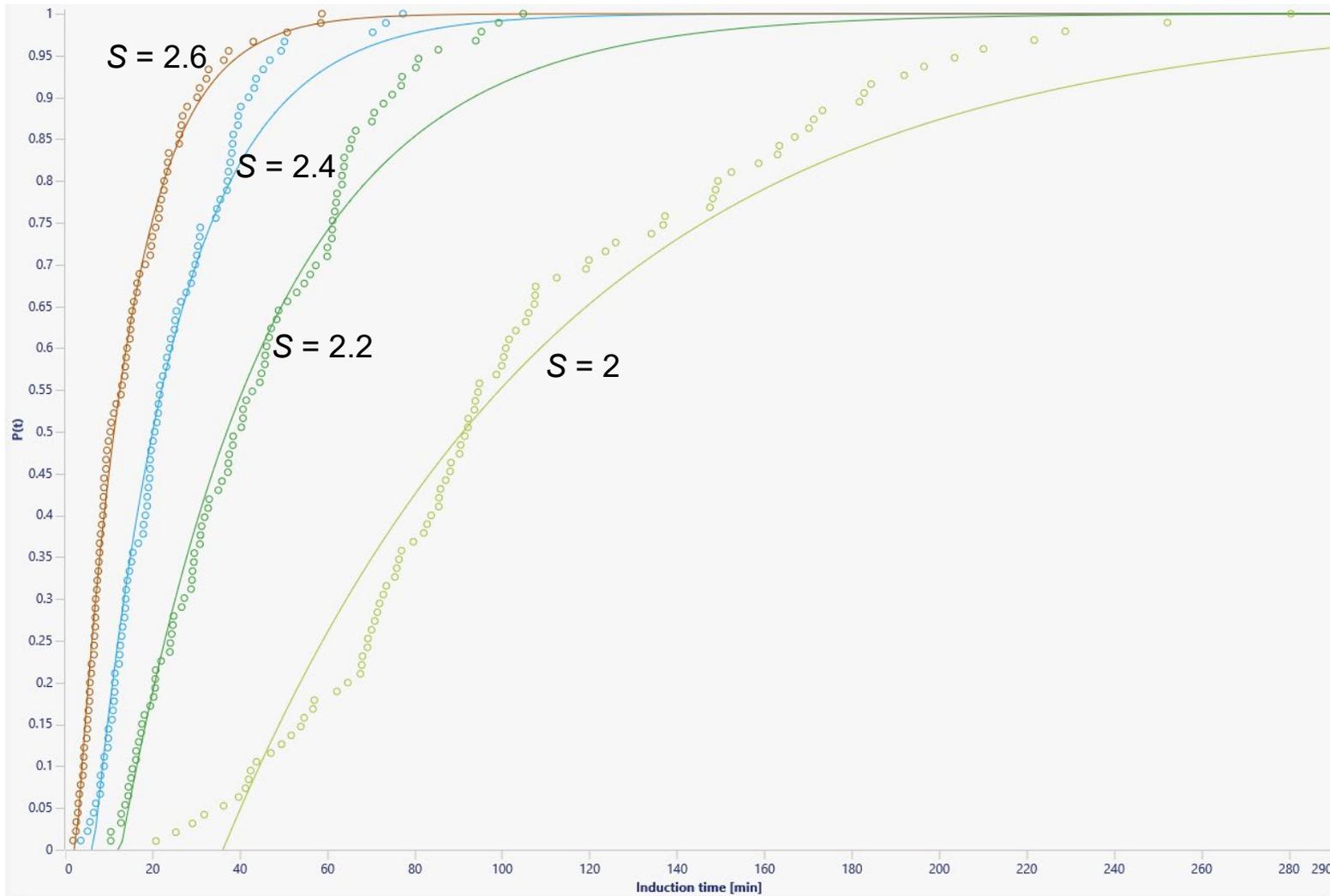


Experimental results

Selecting clear and cloud points



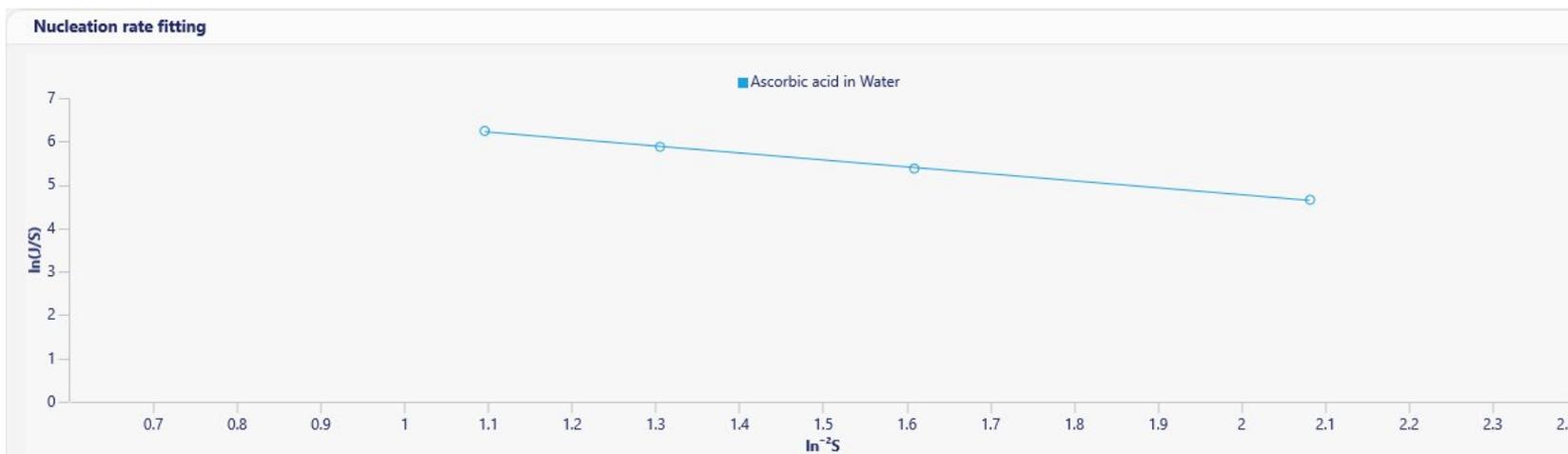
Analysis



$$P(t) = 1 - P_0 = 1 - \exp(-JV(t - t_g))$$

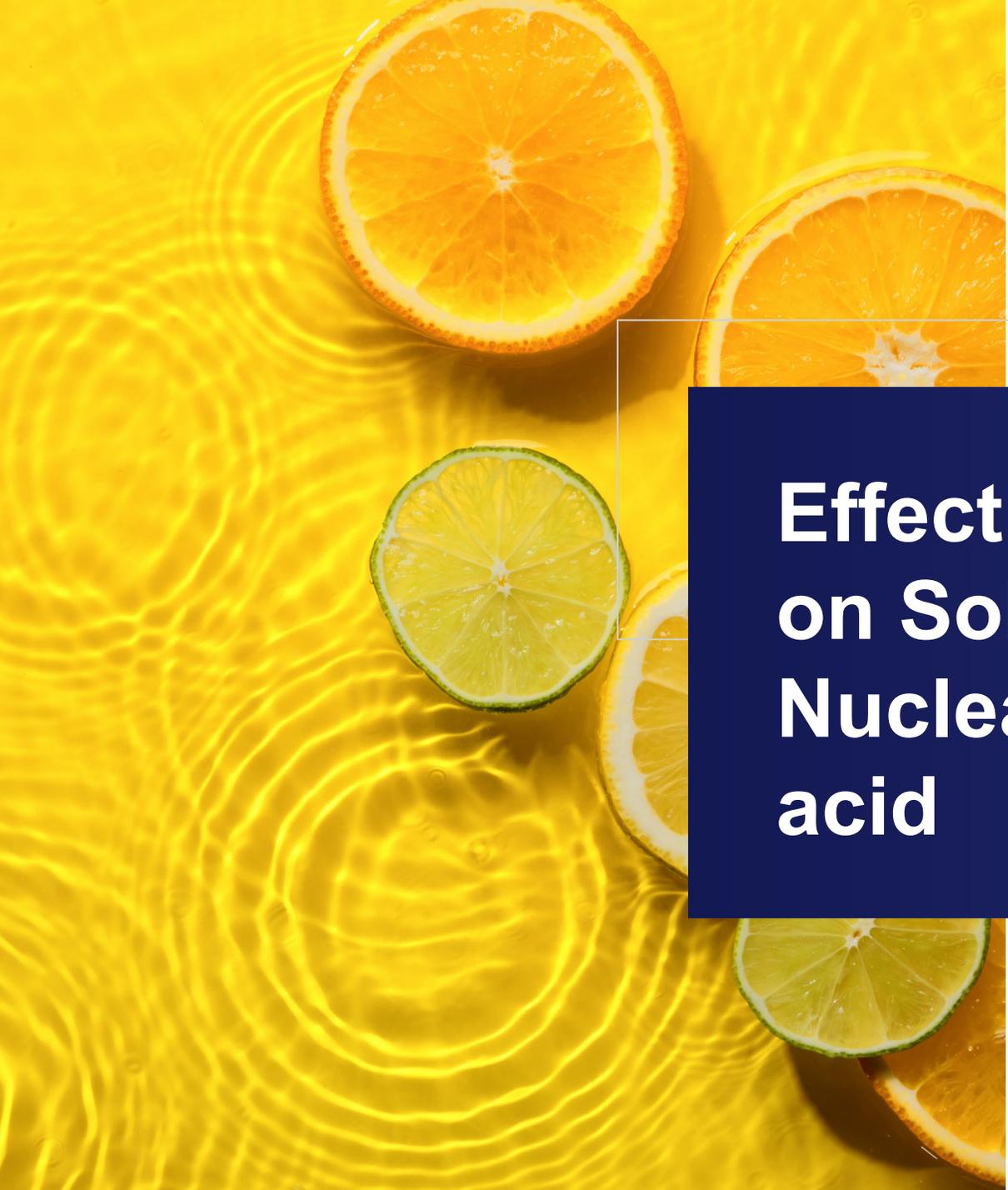
S	J [m⁻³ s⁻¹]
	Water
2	210.92
2.2	491.32
2.4	868.12
2.6	1311.26

Analysis



$$\ln\left(\frac{I}{S}\right) = \ln A - \frac{B}{\ln^2 S}$$

Compound	Solvent	A	B	R ²
Ascorbic acid	Water	2925.04	1.605	0.999

The background of the slide features a vibrant yellow-orange color with a pattern of concentric ripples, suggesting water. Several slices of oranges and limes are scattered across the scene, adding a fresh, citrusy aesthetic. The text is presented in a clean, white, sans-serif font on a dark blue rectangular background that has rounded corners on the right side.

**Effect of solvent composition
on Solubility, MSZW &
Nucleation rate of Ascorbic
acid**

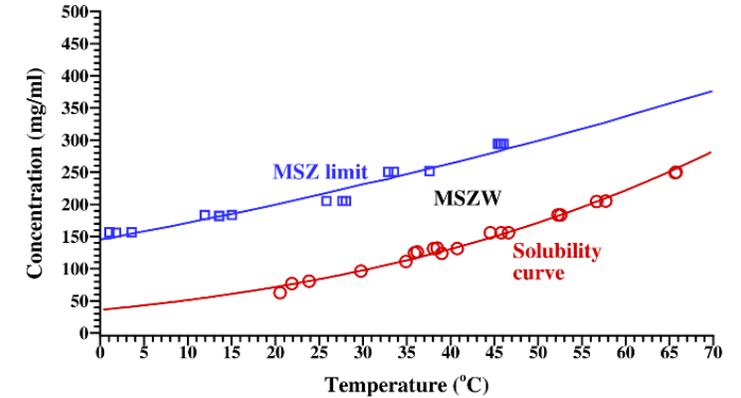
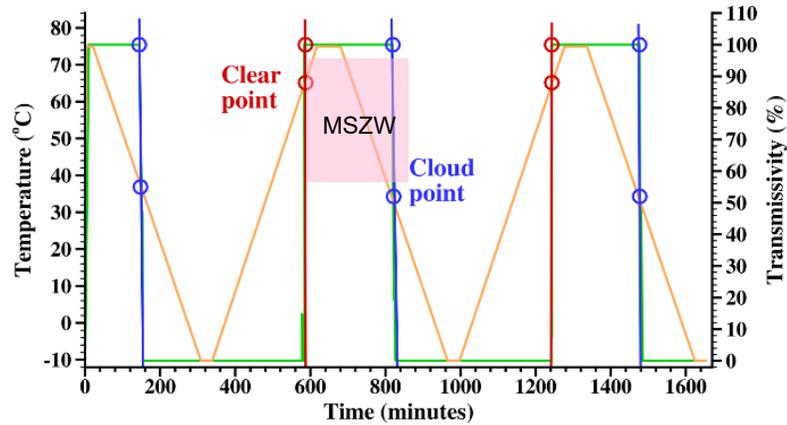
Solubility experiment

Binary solvent system 1		Binary solvent system 2		Binary solvent system 3	
Water	Methanol	Water	Ethanol	Water	Iso-propanol
x_1	x_2	x_1	x_2	x_1	x_2
1	0	1	0	1	0
0.8	0.2	0.8	0.2	0.8	0.2
0.6	0.4	0.6	0.4	0.6	0.4
0.4	0.6	0.4	0.6	0.4	0.6
0.2	0.8	0.2	0.8	0.2	0.8
0	1	0	1	0	1

$$x_2 = \frac{m_2/MW_2}{m_1/MW_1 + m_2/MW_2}$$

X_1 : mole fraction of water

X_2 : mole fraction of alcohol



Operating parameters:

Heating/cooling rate: 0.3 °C/min

Temperature range: -10 °C to 80 °C

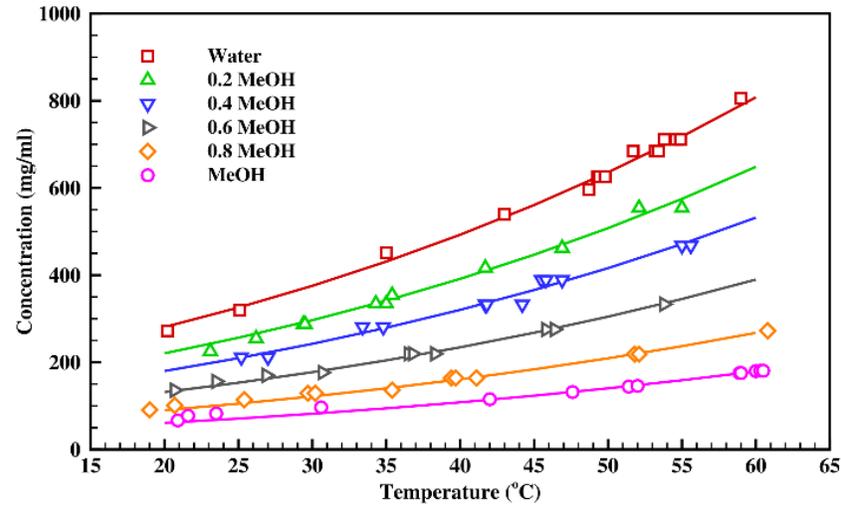
Stirring speed: 600 RPM

Solvent volume: 1 ml

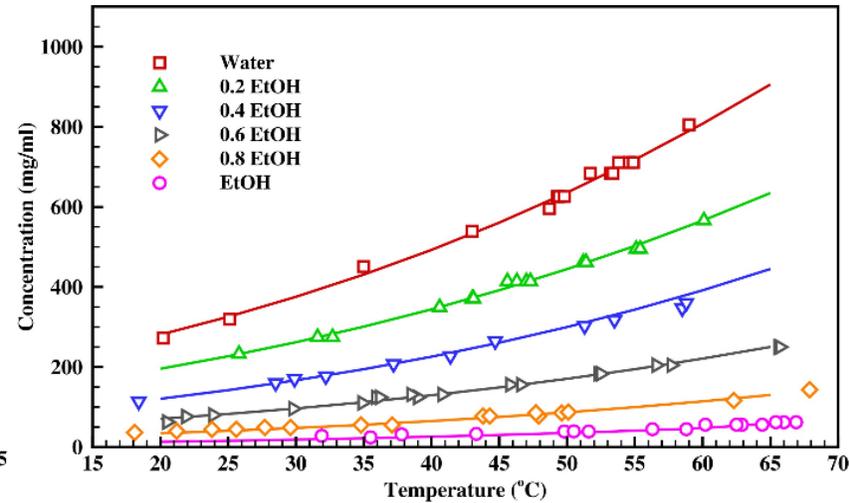
Name	Function	Color	R2
Clear	Van't Hoff	■	0.9952
Cloud	Van't Hoff	■	0.9975

Solubility

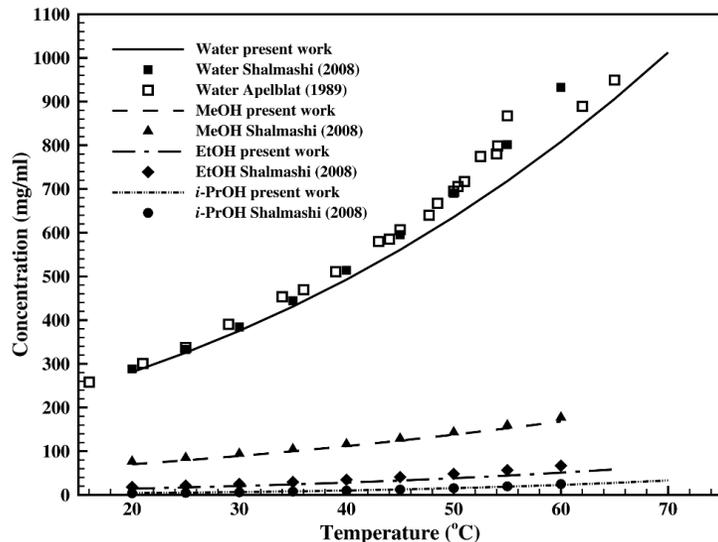
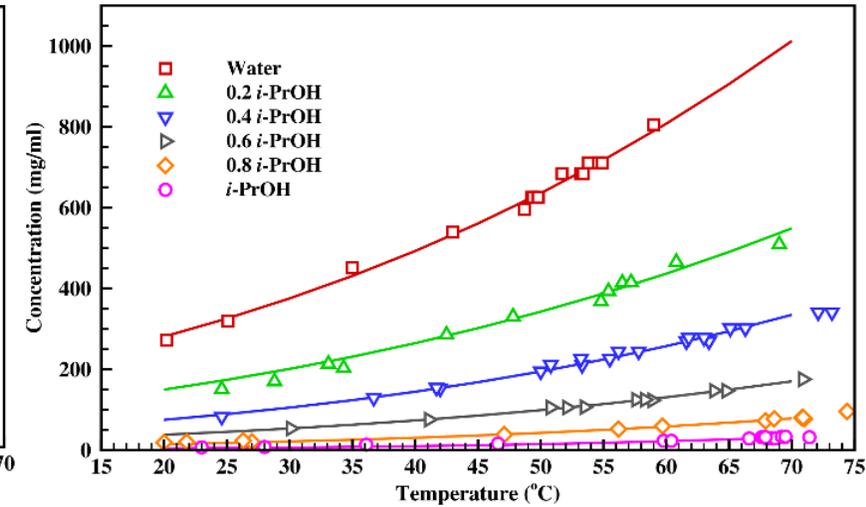
Water-Methanol



Water-Ethanol



Water-Isopropanol



- Solubility **increases** with increase in the **temperature**
- Solubility in pure solvent: **Water > Methanol > Ethanol > Isopropanol**
- With increase in **alcohol concentration** in binary solvent mixture, **solubility decreases**

Yadav, J., Dumitrescu, D. G., Kendall, T., Guguta, C., & Patel, S. A. (2022). *Crystals*, 12(12), 1798.

Shalmashi, A.; Eliassi, A. *J. Chem. Eng. Data* 2008, 53 (6), 1332–1334.

Apelblat, A., & Manzurola, E. *The Journal of Chemical Thermodynamics*, 1989, 21(9), 1005-1008.

Jouyban-Acree model

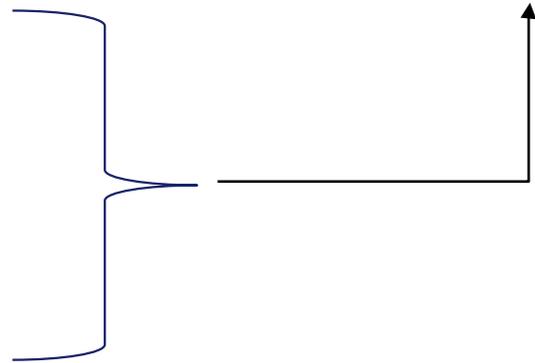
Jouyban-Acree model is commonly used model to predict the solubility in solvent mixtures

$$\ln x_{AA} = x_1 \ln(x_{AA})_1 + x_2 \ln(x_{AA})_2 + \frac{x_1 x_2}{T} \sum_{i=0}^2 J_i (x_1 - x_2)^i$$

$$x_2 = 1 - x_1$$

$$\ln(x_{AA})_1 = a_1 + \frac{b_1}{T}$$

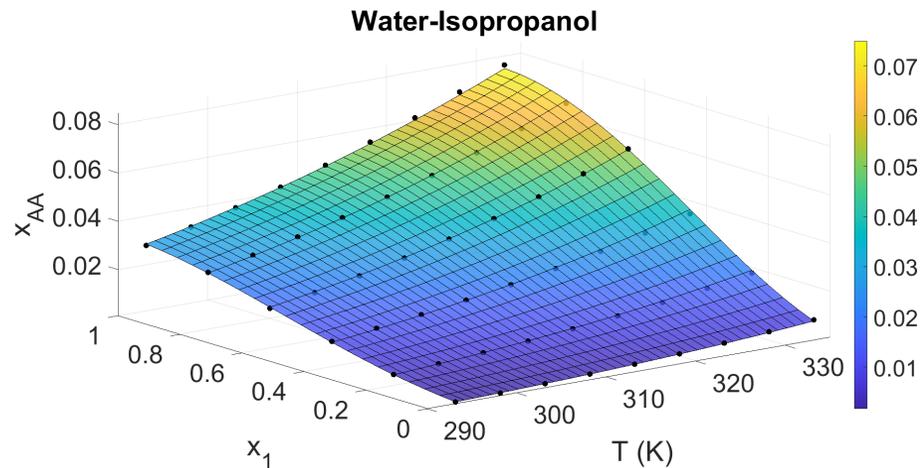
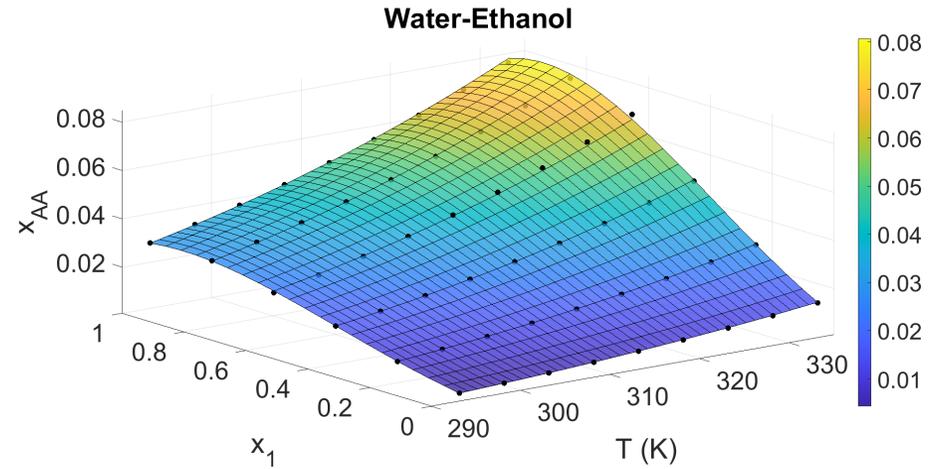
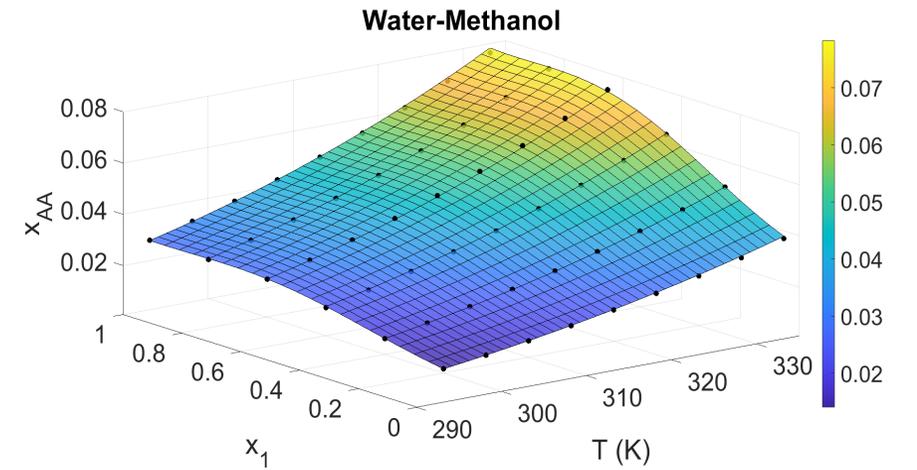
$$\ln(x_{AA})_2 = a_2 + \frac{b_2}{T}$$



$$\ln x_{AA} = A_1 + A_2 \frac{1}{T} + A_3 x_1 + A_4 \frac{x_1}{T} + A_5 \frac{x_1^2}{T} + A_6 \frac{x_1^3}{T} + A_7 \frac{x_1^4}{T}$$

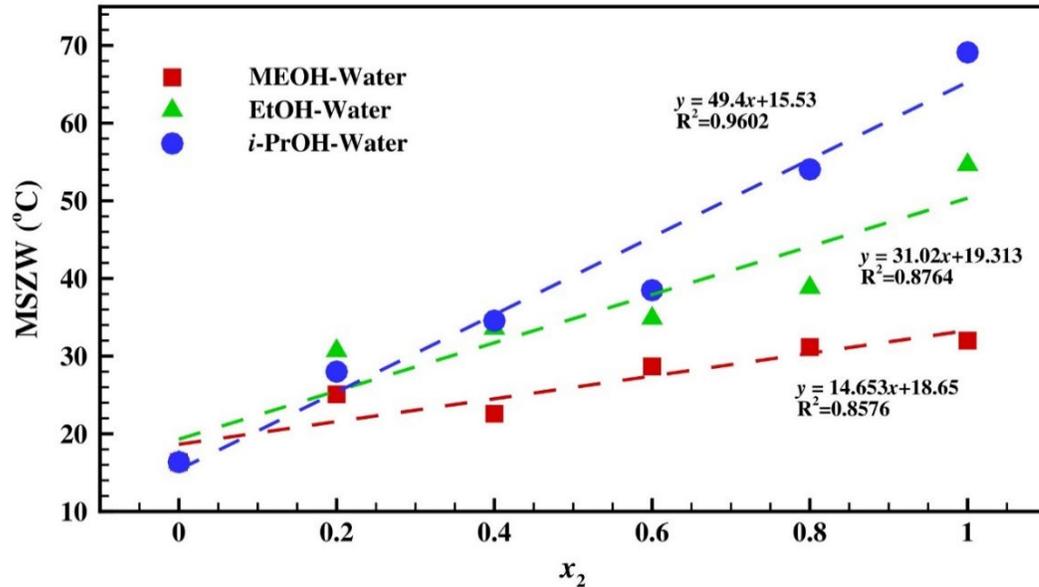
Parameters	Water-MeOH	Water-EtOH	Water- <i>i</i> -PrOH
A ₁	4.691	5.339	7.348
A ₂	-2627	-3164	-4021
A ₃	0.3418	-0.3378	-2.85
A ₄	272.5	1578	2833
A ₅	433.4	-1570	-2027
A ₆	-1215	1085	1229
A ₇	610.6	-444.8	-375.9
R ²	0.9996	0.9997	0.9993
MPD (%)	0.2165	0.2465	0.5033
RMSE	0.0091	0.0128	0.0264

Jouyban-Acree model



Predicting the solubility depending upon two parameters: **temperature** and **solvent composition**

Metastable Zone Width (MSZW)



- MSZW is difference between the **solubility curve** and **MSZ limit**.
- The measurement of MSZW is essential as it reflects the **nucleation kinetics** of the system and defines the optimum supersaturation level required for the crystallization process.
- **MSZW increases** with **increase in alcohol concentration** for all three solvent systems.
- Increase in the MSZW indicates that **higher supersaturation** is required to initiate the primary nucleation.

Results: Nucleation rate

Table: Number of experiments performed in various solvent systems.

Solvent system	Supersaturation, S	Concentration, c, (mg/ml)	No. of data points, N
Water	2.0	561.00	97
	2.2	617.70	104
	2.3	645.36	142
	2.4	674.60	99
	2.6	731.20	93
0.2EtOH+0.8W	2.0	391.19	95
	2.2	430.31	108
	2.3	449.86	119
	2.4	469.43	107
	2.6	508.54	105
0.4EtOH+0.6W	2.2	265.33	91
	2.4	289.51	108
	2.5	301.57	149
	2.6	313.64	82
	2.7	325.76	116
0.6EtOH0.4W	2.3	162.90	101
	2.5	176.20	105
	2.6	183.80	127
	2.7	190.29	103
	2.9	204.90	130

Solvent system	Supersaturation, S	Concentration, c, (mg/ml)	No. of data points, N
Water	2.0	561.00	97
	2.2	617.70	104
	2.3	645.36	142
	2.4	674.60	99
	2.6	731.20	93
0.2iPrOH+0.8W	2.2	329.39	81
	2.3	344.37	114
	2.4	359.34	119
	2.5	374.31	110
	2.6	389.30	101
0.4iPrOH+0.6W	2.4	179.84	84
	2.5	187.33	90
	2.6	194.83	97
	2.7	202.32	88
	2.8	209.82	139
0.6iPrOH+0.4W	2.6	98.88	94
	2.8	106.49	102
	2.9	110.29	88
	3.0	114.10	88
	3.2	121.71	111

Probability plots

$$P(t) = 1 - \exp(-JV(t - t_g))$$

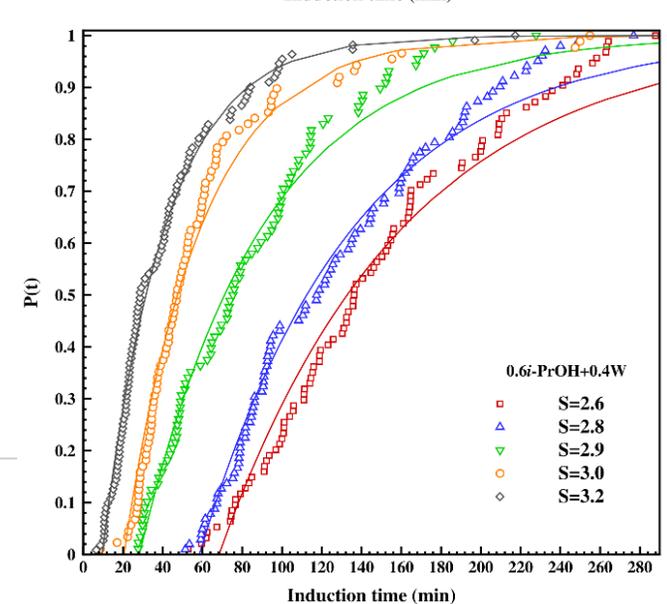
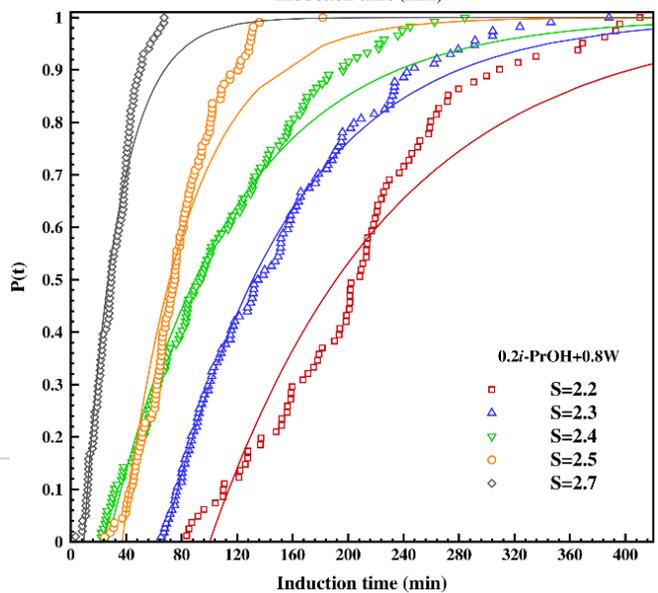
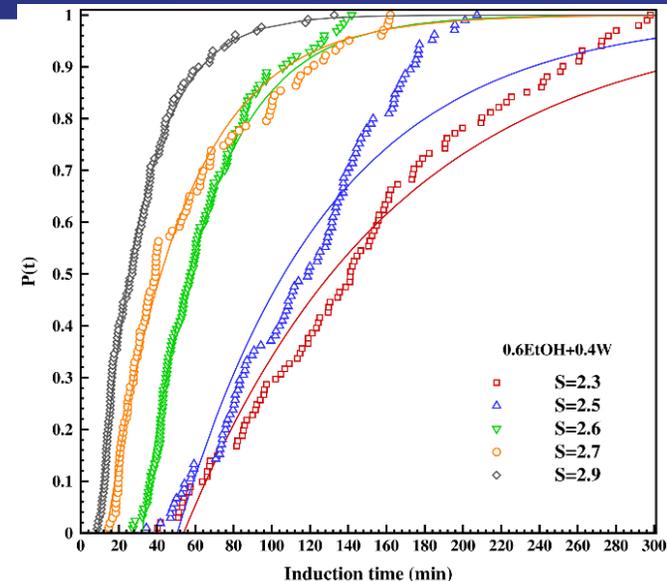
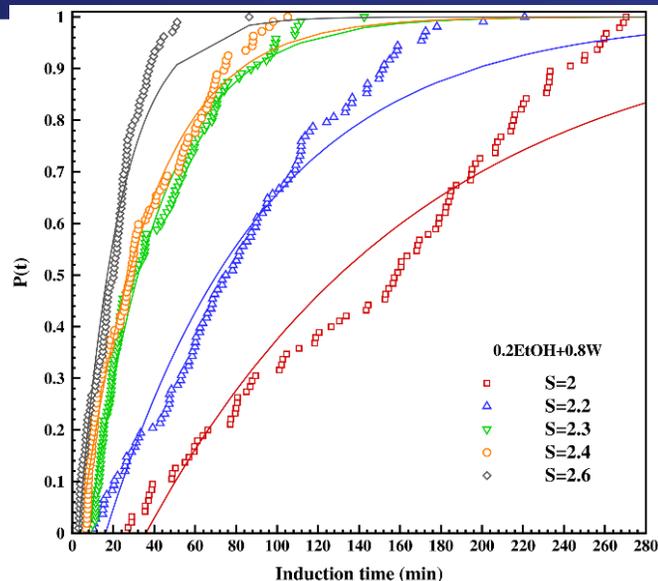
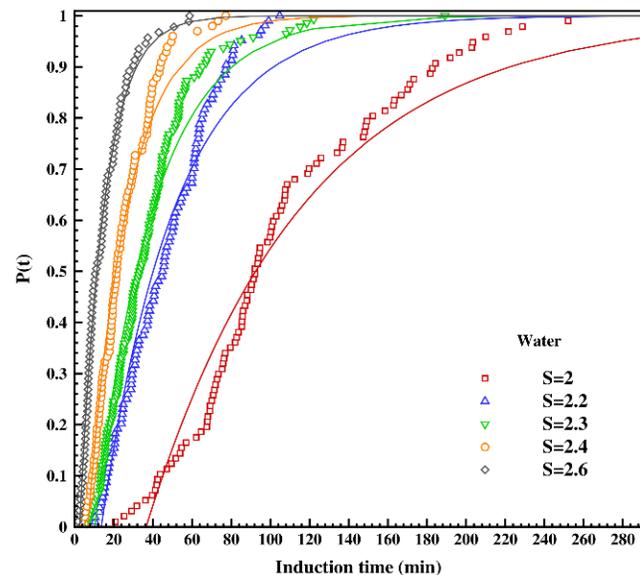


Fig: Probability distribution from experimentally collected induction time data in various water-alcohol solvent systems.

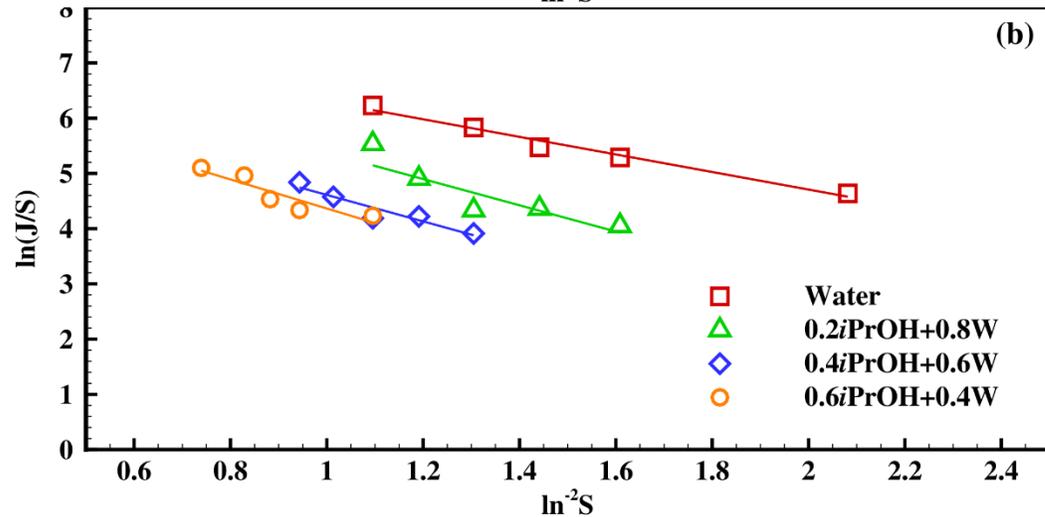
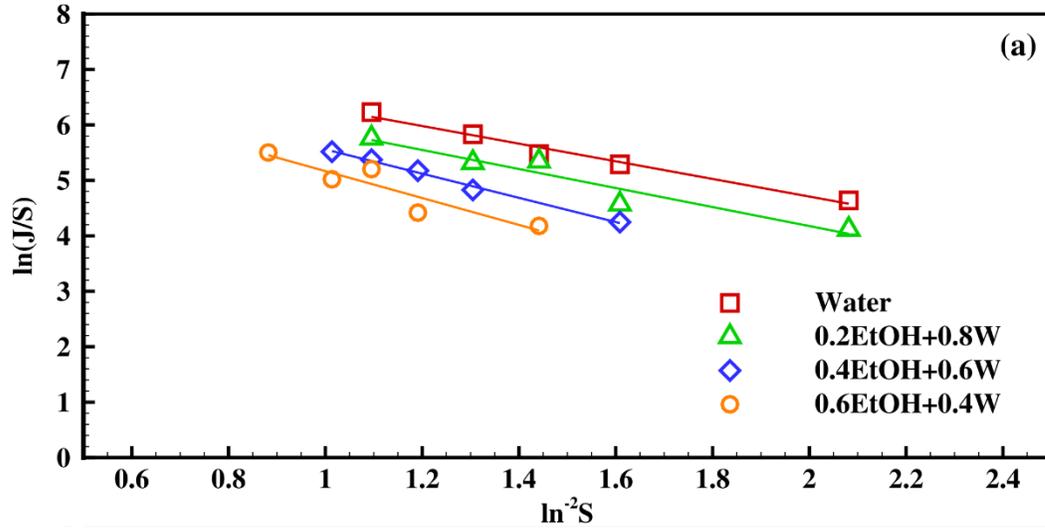
Nucleation rate

Supersaturation, $S = 2.6$

Table: Nucleation rate and growth time for various solvent systems.

Solvent system	Concentration, c, (mg/ml)	No. of data points, N	Nucleation rate, J, (no. /m³sec)	Growth time, t_g (sec)
Water	731.20	93	1319.87	155.56
0.2EtOH+0.8W	508.54	105	825.81	184.79
0.4EtOH+0.6W	313.64	82	559.33	651.32
0.6EtOH+0.4W	183.80	127	472.41	1930.09
0.2i-PrOH+0.8W	389.30	101	658.41	562.80
0.4i-PrOH+0.6W	194.83	97	171.37	2751.20
0.6i-PrOH+0.4W	98.88	94	179.24	4096.49

Nucleation parameters



$$\ln\left(\frac{J}{S}\right) = \ln A - \frac{B}{\ln^2 S}$$

Table: Nucleation kinetic and thermodynamic parameters.

Solvent system	A	B	R ²
Water	2662.48	1.589	0.981
0.2EtOH+0.8W	2008.49	1.715	0.926
0.4EtOH+0.6W	2296.70	2.182	0.993
0.6EtOH+0.4W	1981.20	2.425	0.856
0.2iPrOH+0.8W	2321.18	2.377	0.894
0.4iPrOH+0.6W	1104.98	2.398	0.901
0.6iPrOH+0.4W	1092.94	2.633	0.847

Fig: Plot of $\ln(J/S)$ as a function of $(\ln S)^2$ to determine kinetic parameters of ascorbic acid in: (a) water-ethanol, (b) water-isopropanol solvent systems.

Interfacial energy, Critical radius & Gibbs free energy

Interfacial energy

$$\gamma = \left(\frac{3k^3 T^3 B}{16\pi\vartheta^2} \right)^{1/3}$$

Critical radius

$$r_c = \frac{2\gamma\vartheta}{k_b T \ln S}$$

Critical Gibbs free energy

$$\Delta G_c = \frac{16}{3} \pi \frac{\gamma^3 \vartheta^2}{k_b^2 T^2 (\ln S)^2}$$

Table: Effect of supersaturation on nucleation parameters of ascorbic acid in water.

Supersaturation, S	Thermodynamic parameter, B	Interfacial energy, γ , (mJ/m ²)	Critical radius, r_c , (Å ⁰)	Gibbs free energy, ΔG_c , (kJ/mol)
2.0	1.589	5.544	7.327	7.503
2.2			6.441	5.799
2.3			6.097	5.196
2.4			5.801	4.703
2.6			5.315	3.948

Table: Nucleation parameters for ascorbic acid in various solvent systems. (S=2.6)

Solvent system	Thermodynamic parameter, B	Interfacial energy, γ , (mJ/m ²)	Critical radius, r_c , (Å ⁰)	Gibbs free energy, ΔG_c , (kJ/mol)
Water	1.589	5.544	5.315	3.948
0.2EtOH+0.8W	1.715	5.687	5.452	4.261
0.4EtOH+0.6W	2.182	6.162	5.908	5.422
0.6EtOH+0.4W	2.425	6.383	7.020	7.930
0.2iPrOH+0.8W	2.377	6.340	6.079	5.906
0.4iPrOH+0.6W	2.398	6.359	6.096	5.958
0.6iPrOH+0.4W	2.633	6.560	6.289	6.542

Summarizing...

- Crystal16 is well known for measurement of solubility and MSZW.
- **Crystal16V3** comes with new features for end user benefits.
- Crystal16 for all crystallization applications.
- We learned how to determine the **Nucleation rate** with Crystal16V3.
- Nucleation rate of Ascorbic acid in various solvent systems:
 - Ascorbic acid has high nucleation rate in water.
 - The **thermodynamic parameter B** increases and **kinetic parameter A** decreases with an increase in alcohol composition.
 - The **critical radius** and **Gibbs free energy** were determined from the thermodynamic parameter B , which increases with increase in alcohol composition.

Thank you!!



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