Crystallization from solutions

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Thermodynamics & Kinetics of crystallization - nucleation

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Thermodynamically stable phases of organic and inorganic substances depend on:

- Temperature
- Pressure
- surrounding media:

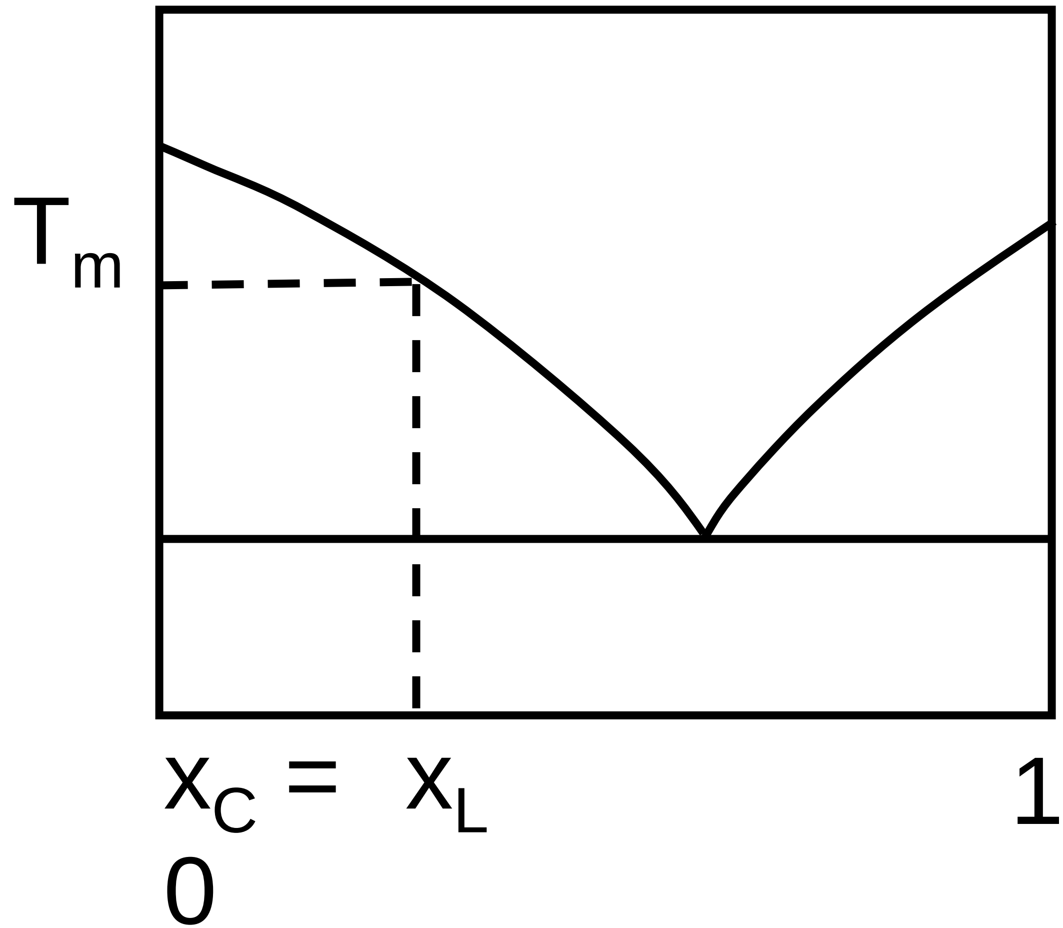
  - air (relative humidity)
  - solvent (solubility)

- additional: mechanical stress (e.g. grinding)

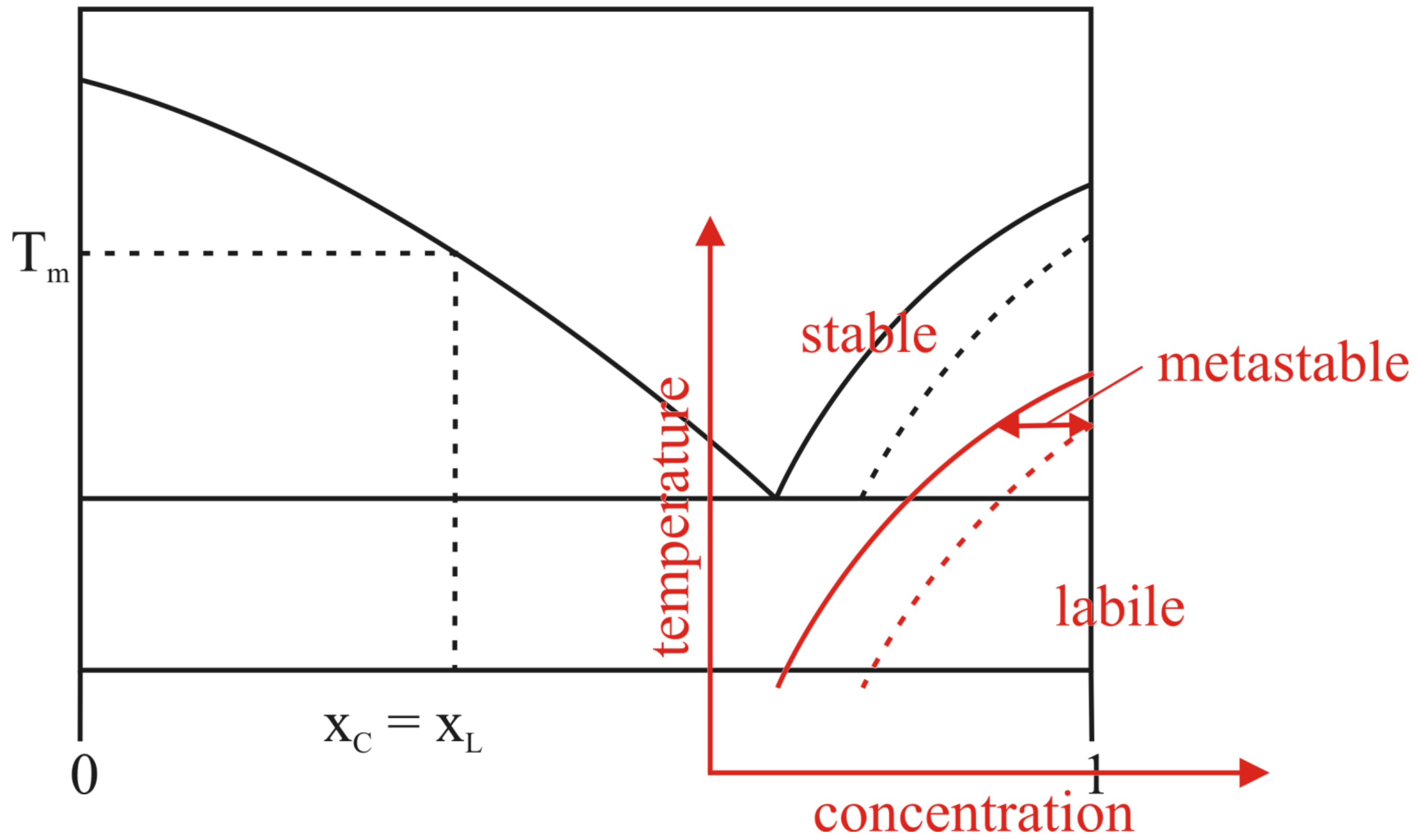
Phase diagrams

“A phase diagram graphically represents (in two or three dimensions) the equilibria between various phases of a system in a wide range of temperature, pressure and concentration/composition.
It specifies the equilibrium conditions (T, p and x) and the corresponding phase present at this state.
Thus, in case of SLE, the phase diagram also tells about the solid phases occurring in a system, such as polymorphs, solvates or intermediate compounds” [LOR13].

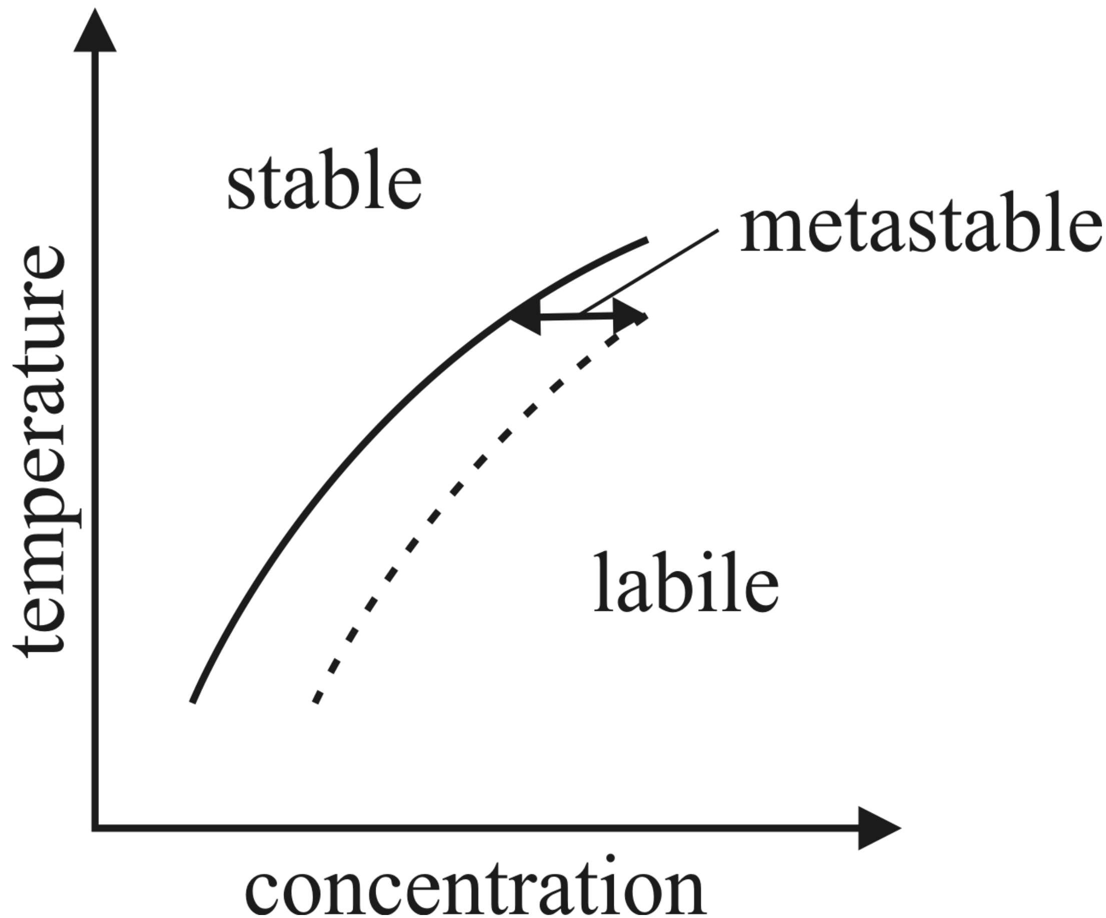
Phase diagram - melts



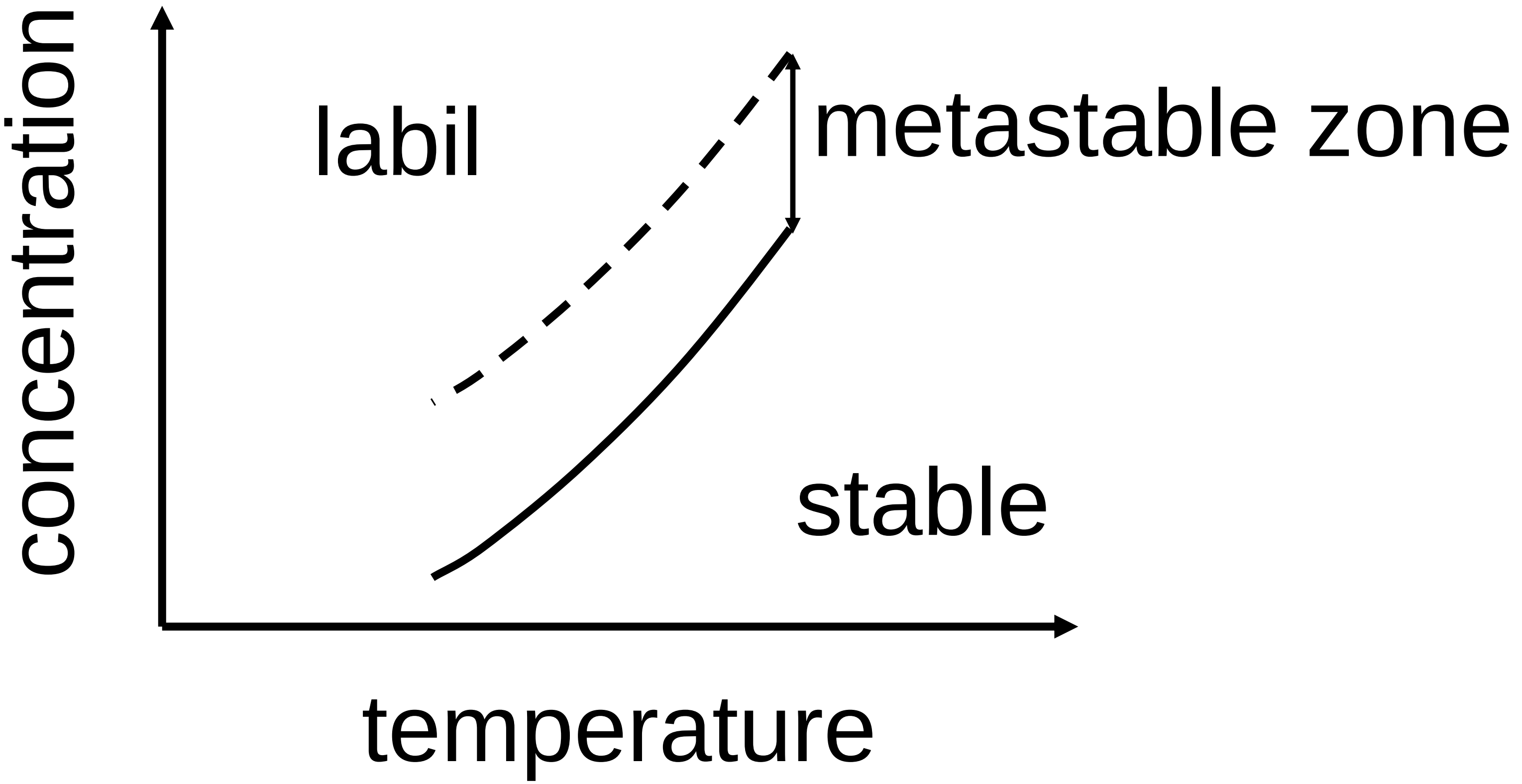
Phase diagram overlapped by the „wrong plotted“ solubility diagram

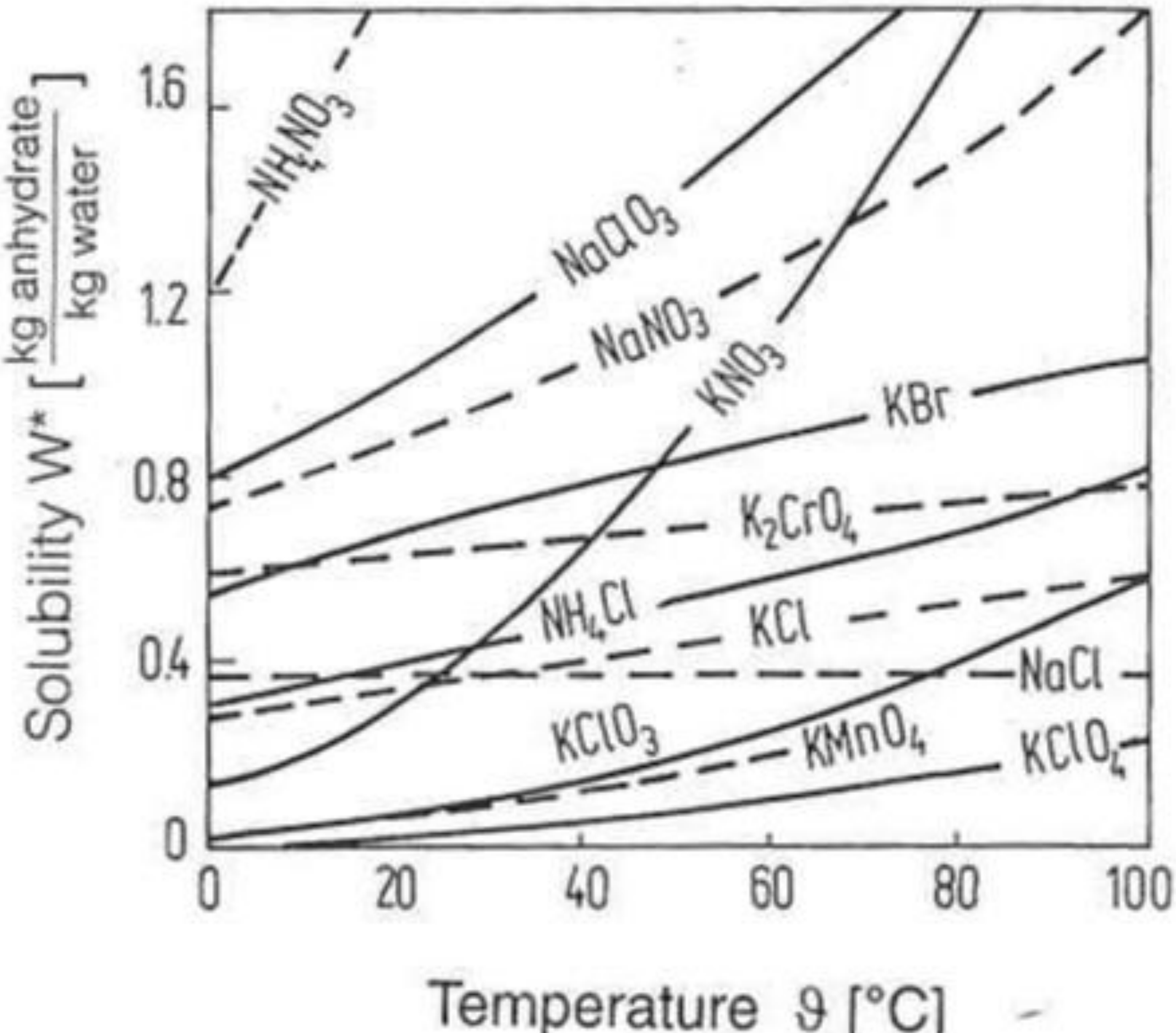


Solubility diagram – „wrong plotted“



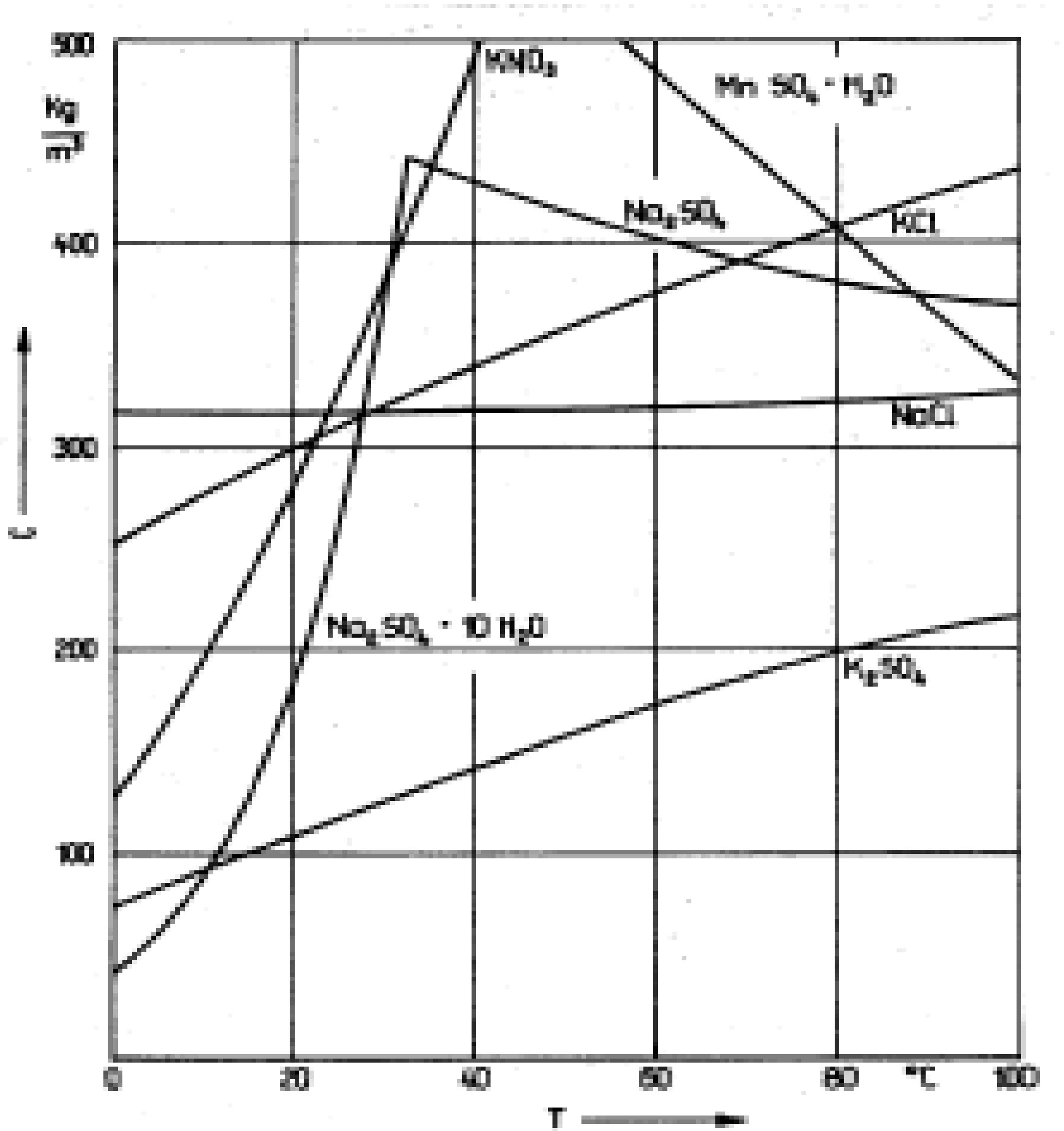
Solubility diagram - solutions

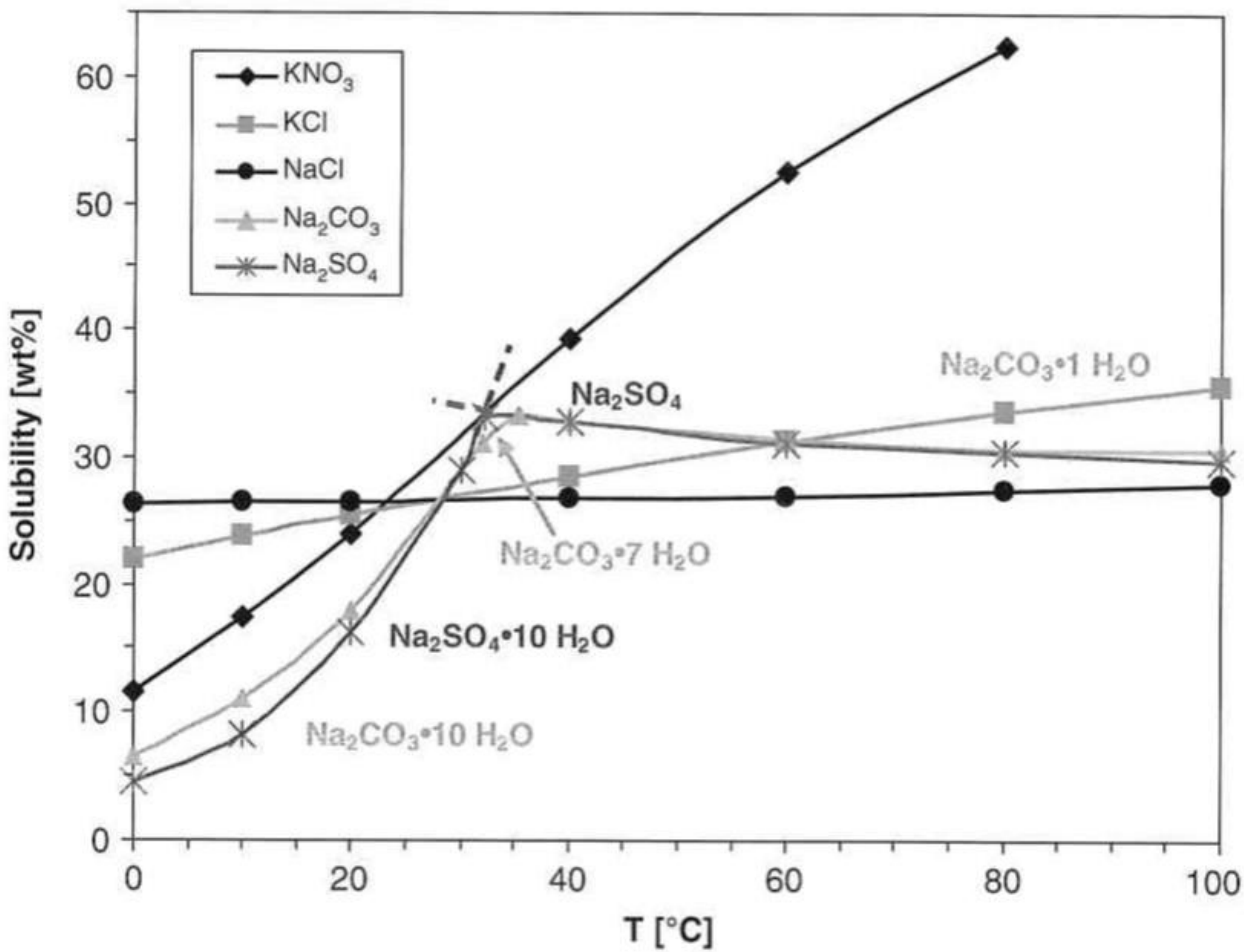




Solubility W* [kg anhydrate / kg water]
1.6
1.2
0.8
0.4
0
NH4NO3
NaClO3
NaNO3
KNO3
KBr
K2CrO4
NH4Cl
KCl
NaCl
KClO3
KMnO4
KClO4
0
20
40
60
80
100
Temperature ϑ [°C]

Temperature-Solubility-Diagram





KNO3
KCl
NaCl
Na2CO3
Na2SO4
Na2CO3•1 H2O
Na2SO4
Na2CO3•7 H2O
Na2SO4•10 H2O
Na2CO3•10 H2O
Solubility [wt%]
T [°C]
0
10
20
30
40
50
60
0
20
40
60
80
100

kg hydrate / kg solution

kg anhydrous / kg solution

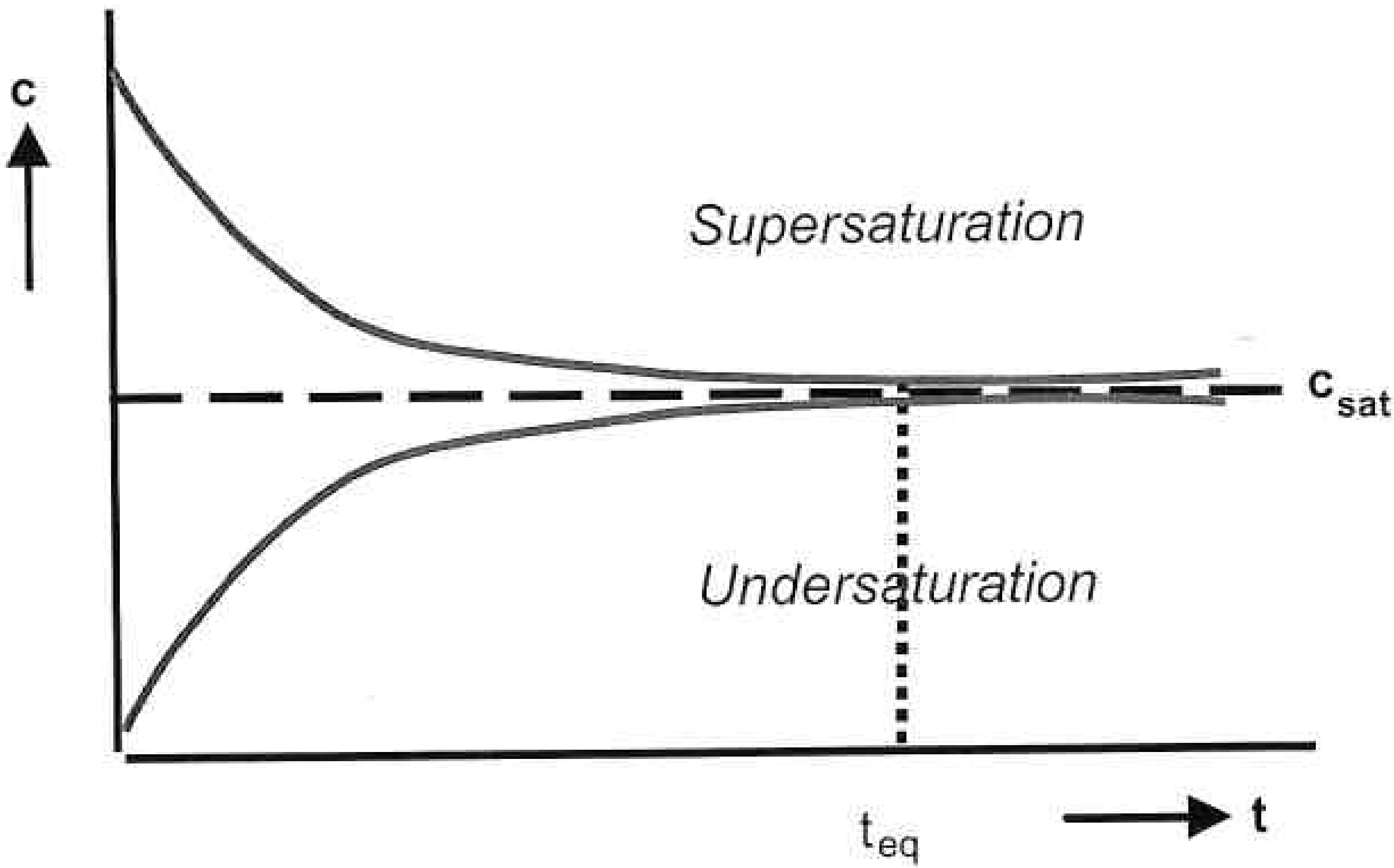
kg hydrate / kg solvent

kg anhydrous / kg solvent

kg hydrate / m^3 solvent

kg hydrate / m^3 solution

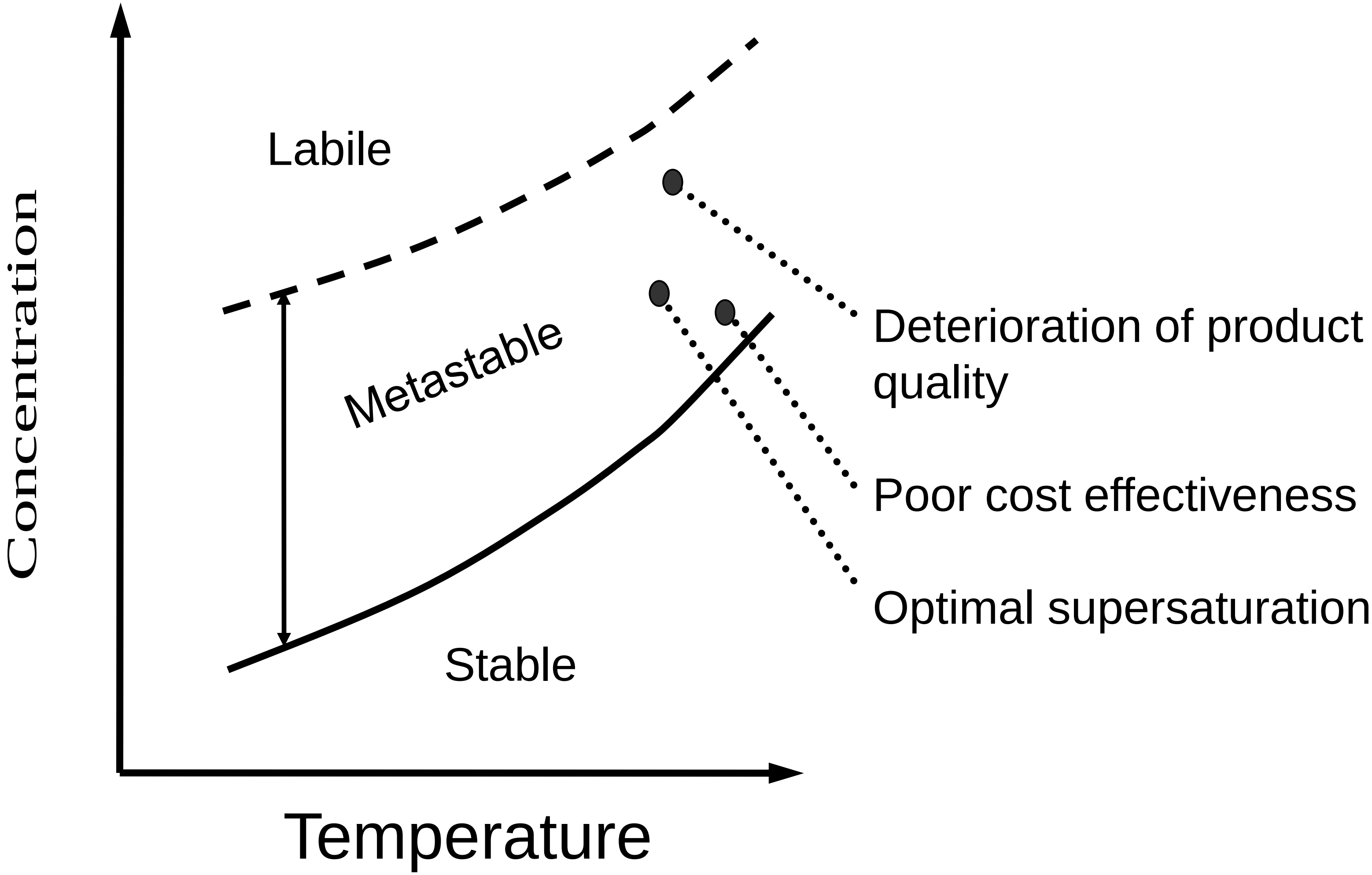
mole hydrate / mole solution etc.


c
Supersaturation
c_sat
Undersaturation
t_eq
t

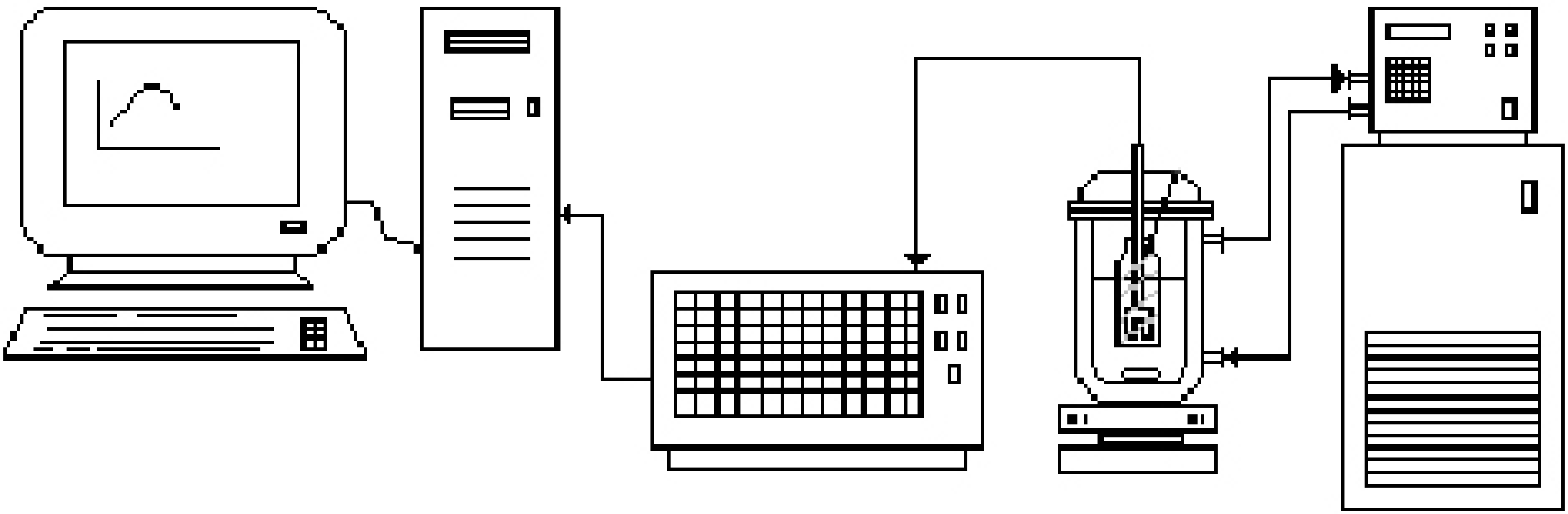
Metastable zone width

- What is it ?
- How to determine ?
- How to influence ?
- Why important ?

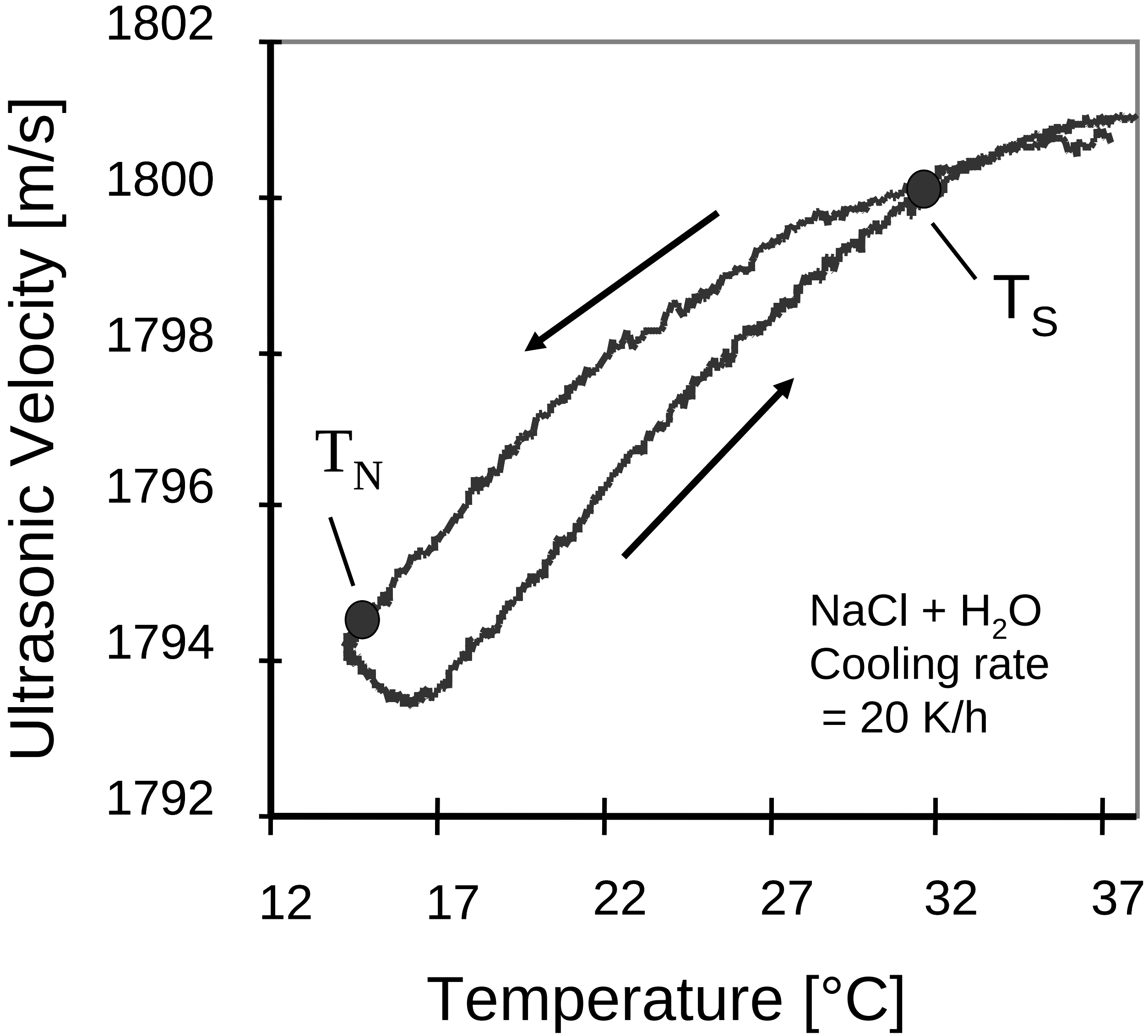
Metastable zone



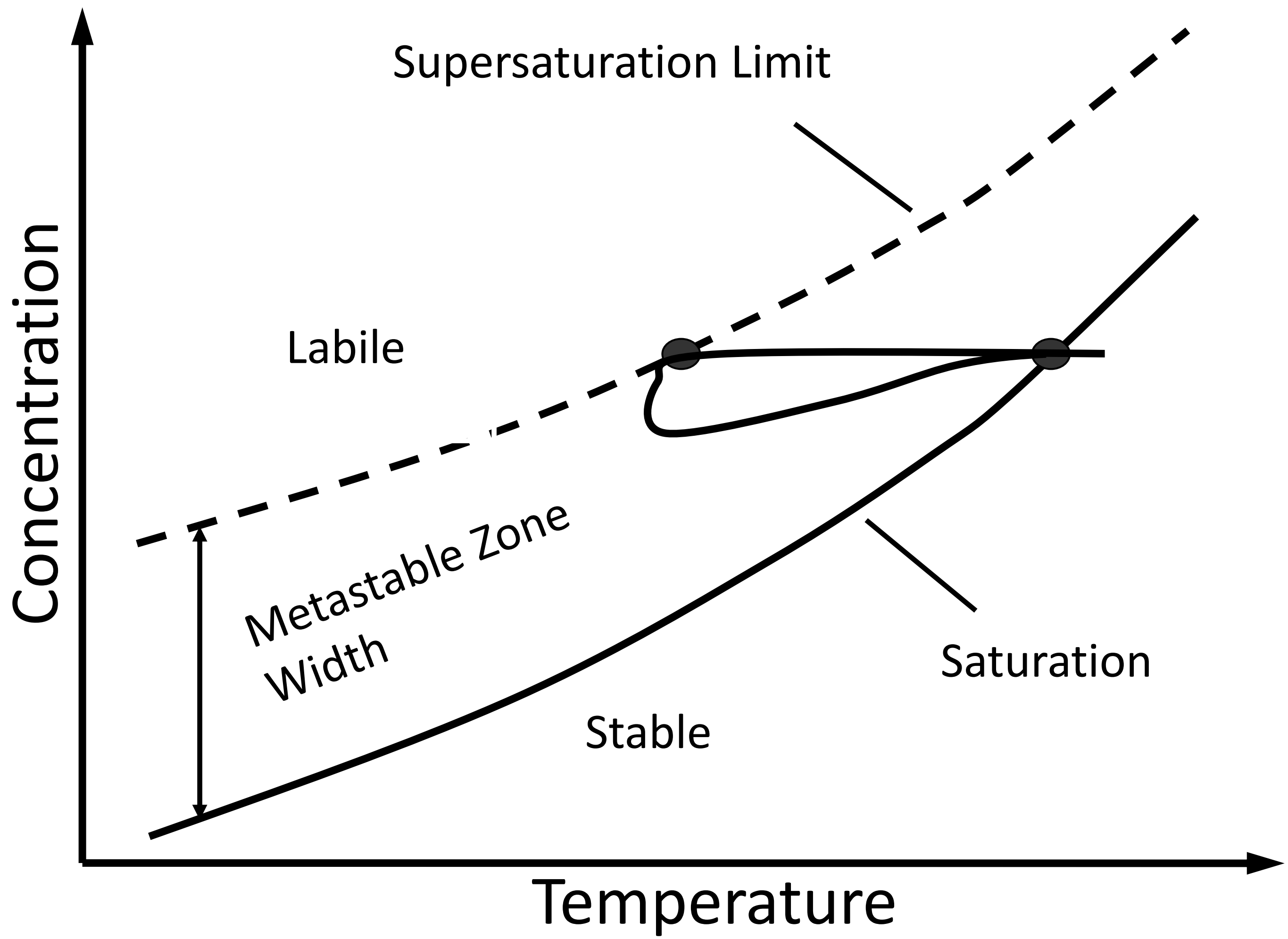
In line process control ultrasonic sensor



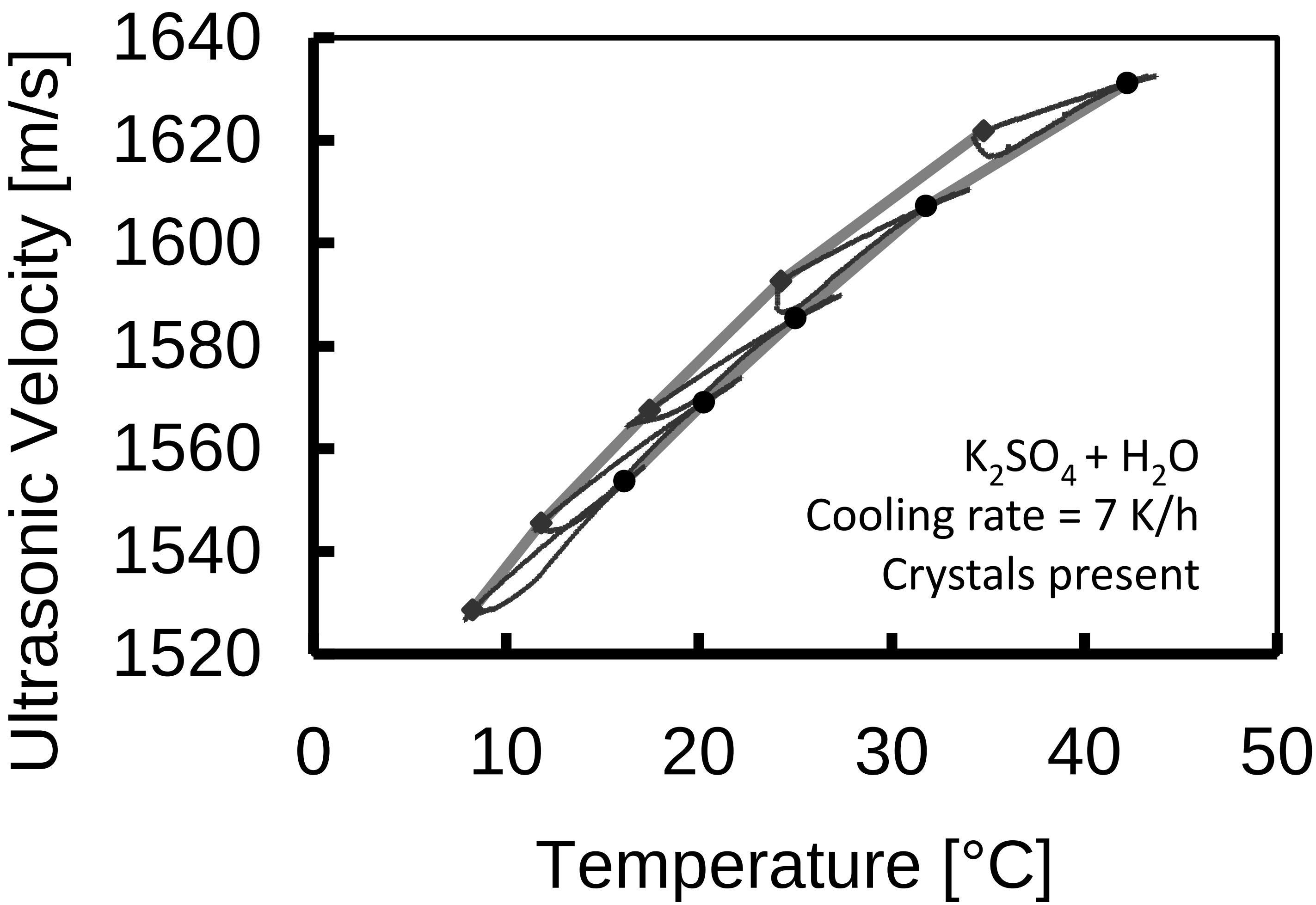
Process parameters



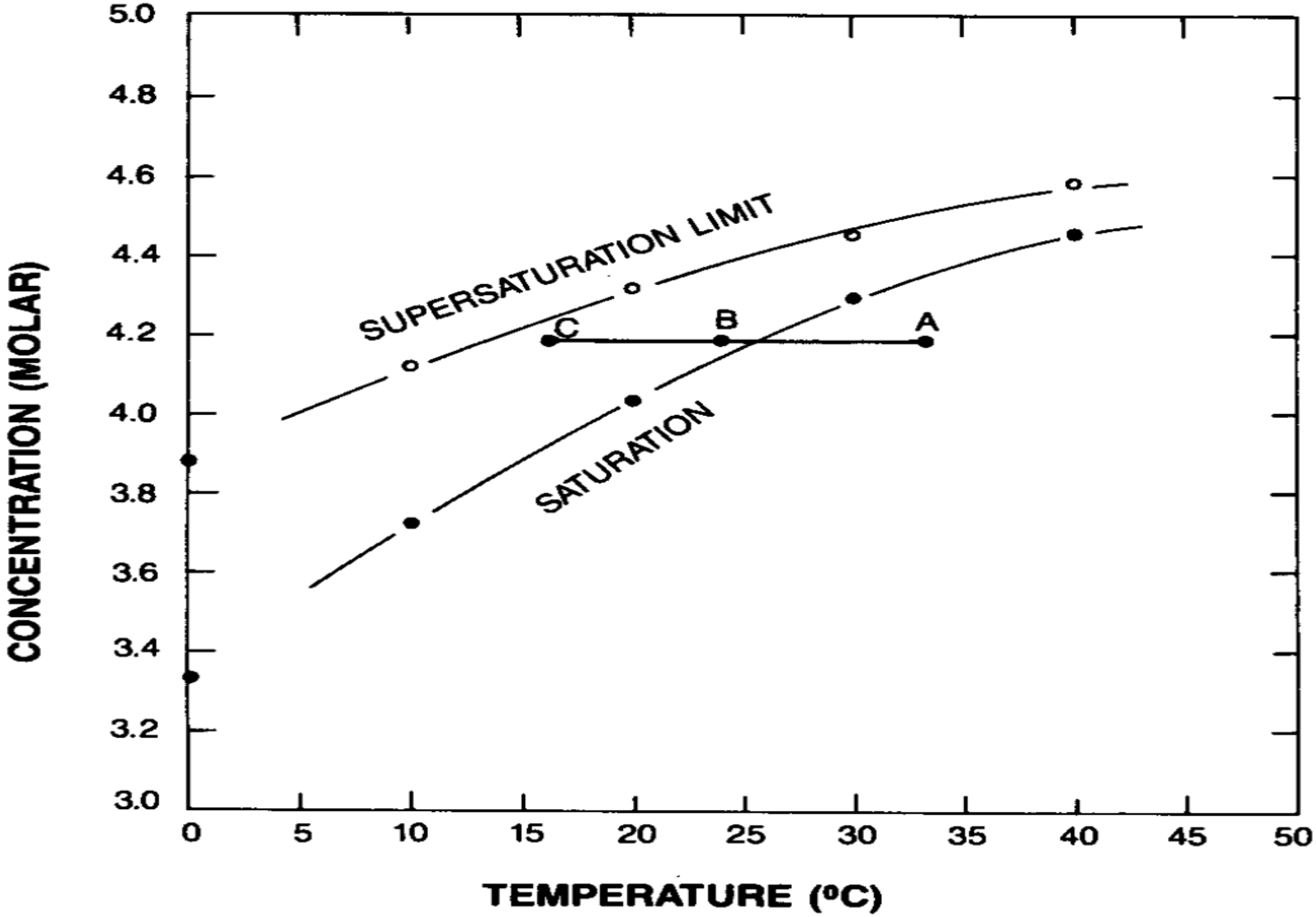
Determination of metastable zone width I



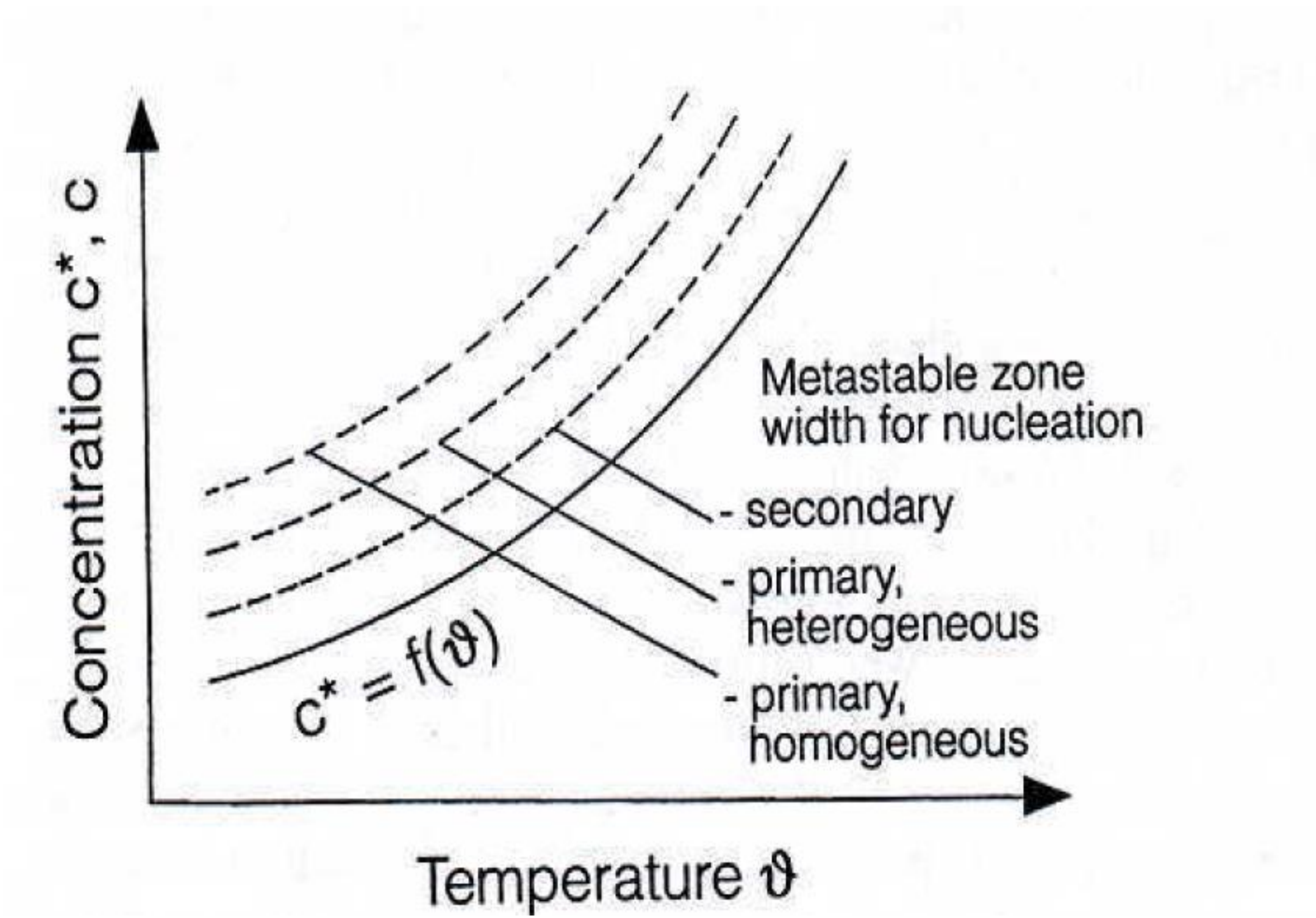
Determination of metastable zone width II



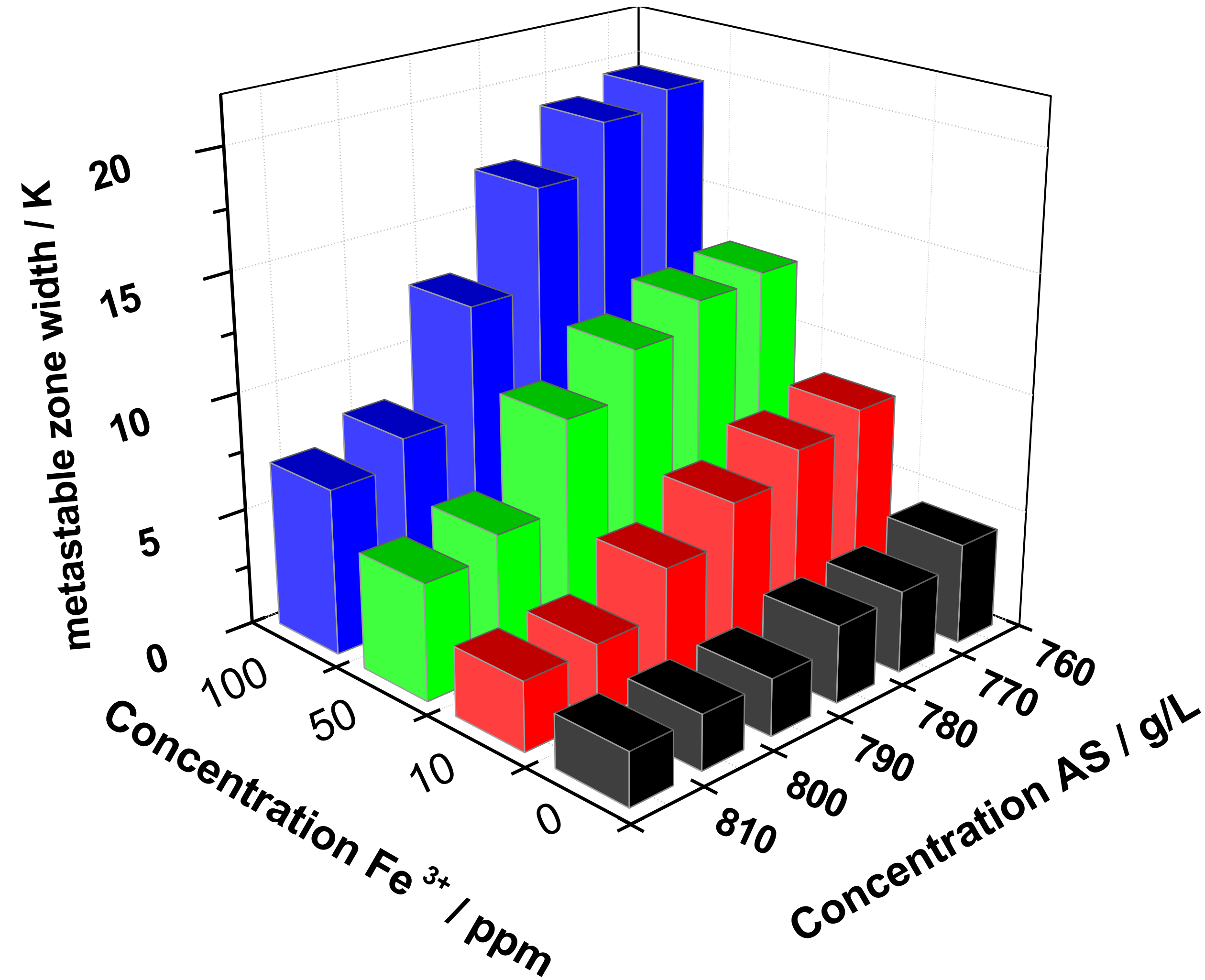
Metastable zone width for KCl-water system (Chang 1984)



Metastable zone width for nucleation



Effects on Metastable Zone Width

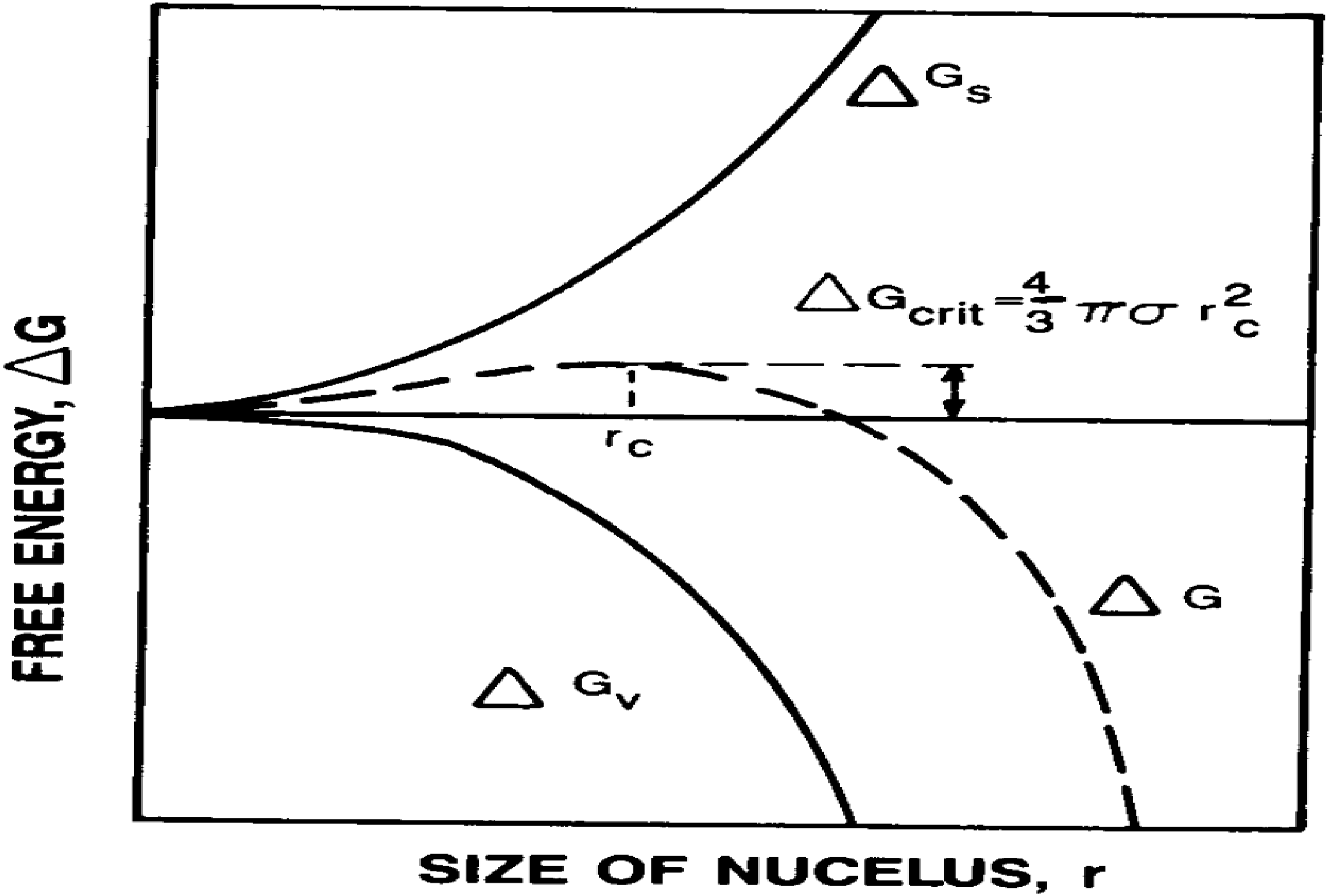


Effect of Fe^{3+} on the metastable zone width of ammonium sulfate at pH 4

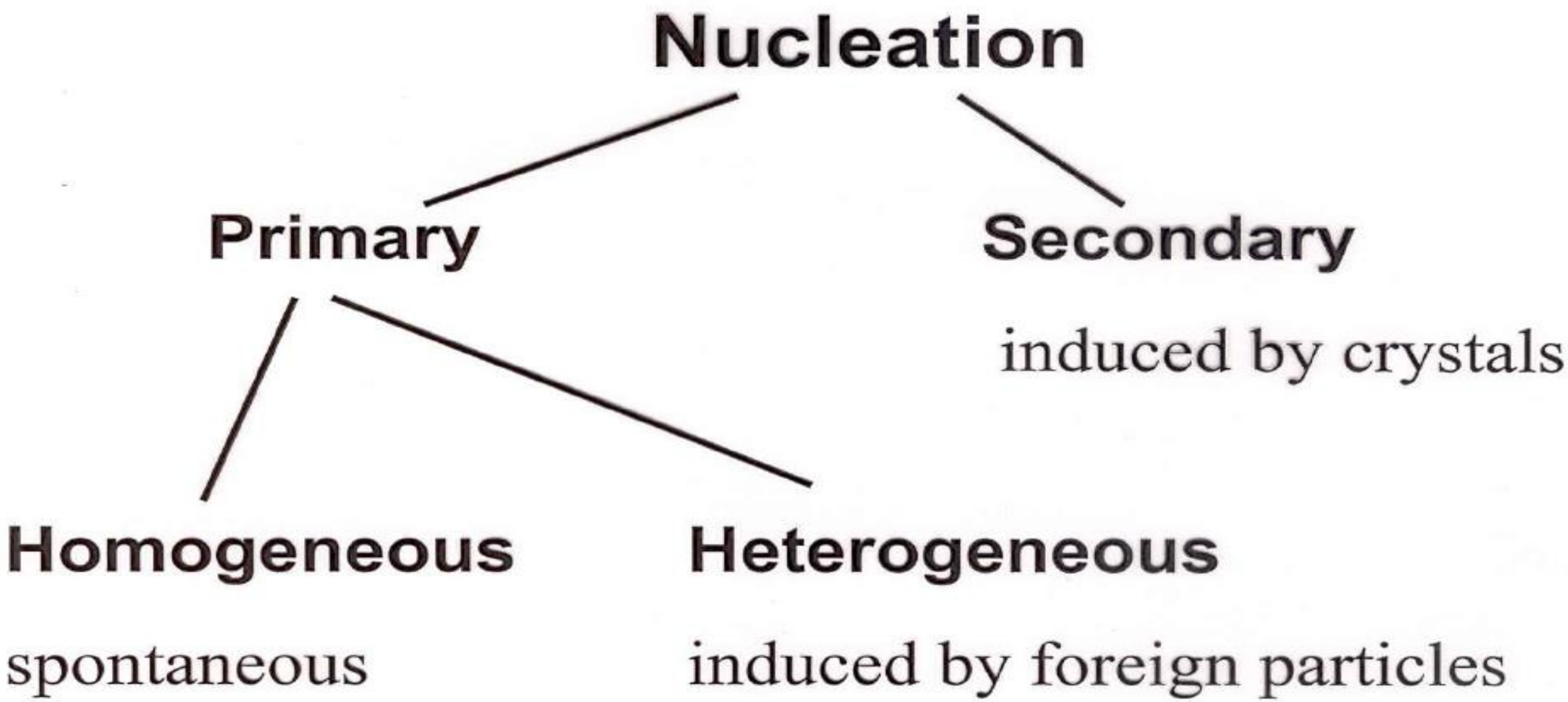
Solute	Dimensionless solubility C^*/C_C	Solubility parameter $\frac{d(\ln C^*)}{d(\ln T)}$	Maximum detectable subcooling $\Delta T_{met,exp}$ in K for $dT/dt =$ 2 K/h	5 K/h	20 K/h
Calcium nitrate	0.71	2.0	0.6	0.9	1.5
Boric acid	0.04	7.9	1.3	1.9	3.1
Potassium bromide	0.20	1.4	1.1	1.6	2.7
Potassium iodide	0.33	0.6	0.6	0.8	1.2
Potassium nitrite	0.62	0.3	0.9	1.2	1.8
Potassium nitrate	0.18	7.0	0.4	0.5	0.7
Sodium sulfite	0.39	6.6	0.56	0.6	0.7
Sodium bromide	0.48	1.2	1.0	1.4	2.3
Sodium iodide	0.64	0.7	1.2	1.4	2.0
Sodium nitrite	0.29	1.2	1.4	1.8	2.6

No.	Solute	Formula	Molar mass (Anh/Hyd) $\tilde{M}$ [kg/kmol]	h_{20} [-]	Density (Hydrate) ρ_c [kg/m³]	 C_c [kmol/m³]	Mass ratio (Hydrate) W^*_{20} [kg_{Hyd}/kg_{H2O}]	Density $\rho^*_{L,20}$ [kg/m³]	Solubility (Hydrate) C^*_{20} [kmol/m³]	C^*/C_c [-]	Heat of crystall. ΔH_{LC} [kJ/mol]	No.
	ALUMINIUM											
1	chloride	$AlCl_3$	133.34 241.432	6	2398	9.93	1.3068	1492	3.500	0.35	-13	1
2	hydroxide	$Al(OH)_3$	77.99	0	2420	31.03	0.00012	998	0.002	5.0E-5		2
3	sulphate	$Al_2(SO_4)_3$	342.134 630.379	16	1690	2.68	1.0017	1321 1255	0.997	0.37	-64	3
	AMMONIUM											
4	aluminium sulphate	$(NH_4)_2Al_2(SO_4)_4$	474.306 906.672	24	1640	1.81	0.1107	1050 1039	0.115	0.06		4
5	bromide	NH_4Br	97.942	0	2429	24.80	0.755	1300 1337	5.710	0.23	-15	5
6	chloride	NH_4Cl	53.491	0	1527	28.55	0.372	1076 1102	5.454	0.19	16.2	6
7	dihydrogen phosphate	$NH_4H_2PO_4$	115.025	0	1803	15.67	0.365	1160 1134	2.697	0.17		7
8	ferrous sulphate	$(NH_4)_2Fe(SO_4)_2$	284.038 392.13	6	1864	4.75	0.3798	1171 1145	0.803	0.17		8
9	hydrogen carbonate	NH_4HCO_3	79.055	0	1580	19.99	0.21	1066	2.341	0.12		9
10	nickel sulphate	$(NH_4)_2Ni(SO_4)_2$	286.908 395	6	1923	4.87		1052				10
11	nitrate	NH_4NO_3	80.043	0	1725	21.55	1.92	1310 1381	10.761	0.50	-12	11

Free energy versus cluster radius (Mullin 1972)

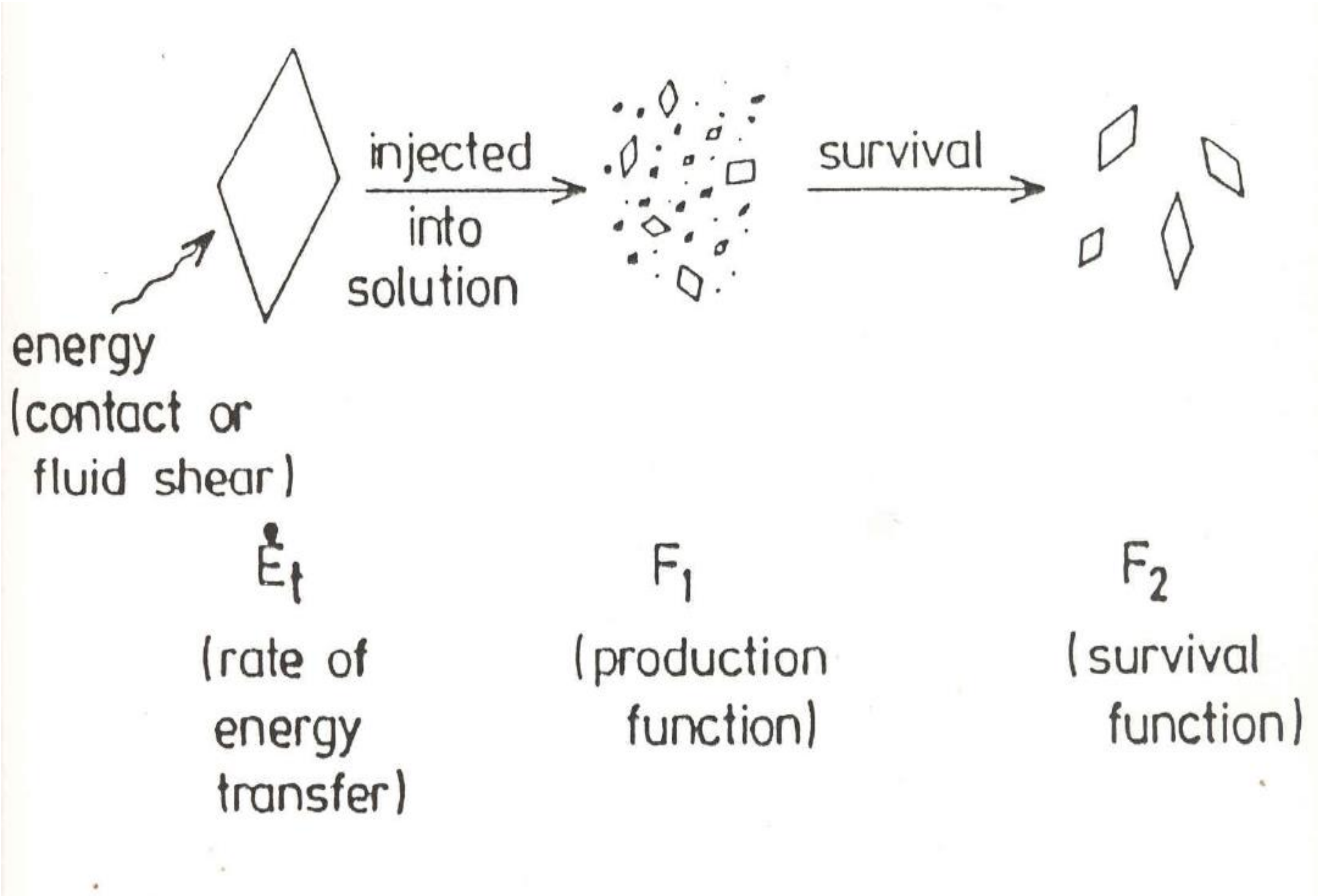


Mechanisms of Nucleation

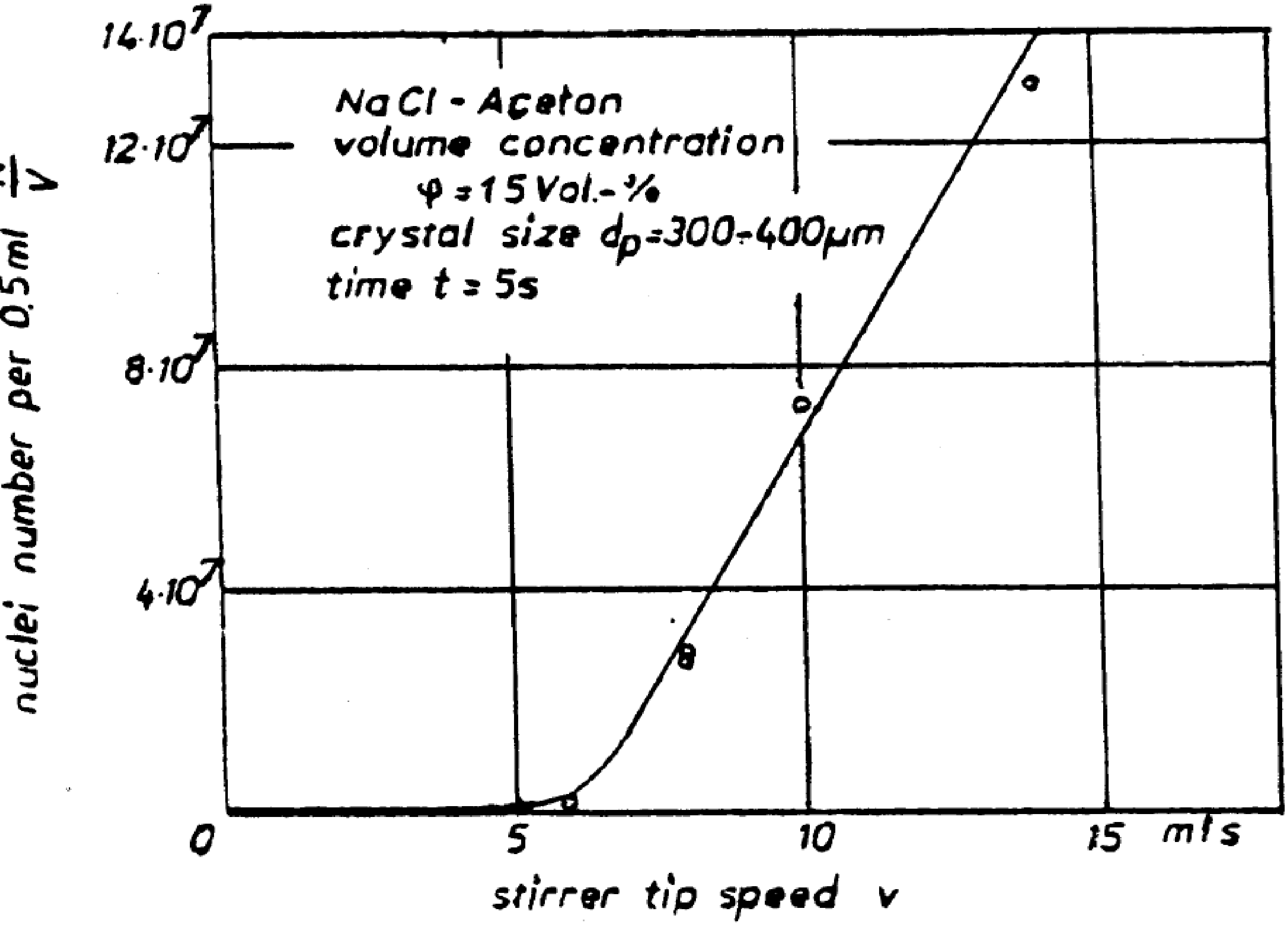


Supersaturation S	Time
1.0	Infinity
2.0	10^{60} years
3.0	10^{3} years
4.0	0.1 second
5.0	10^{-13} second

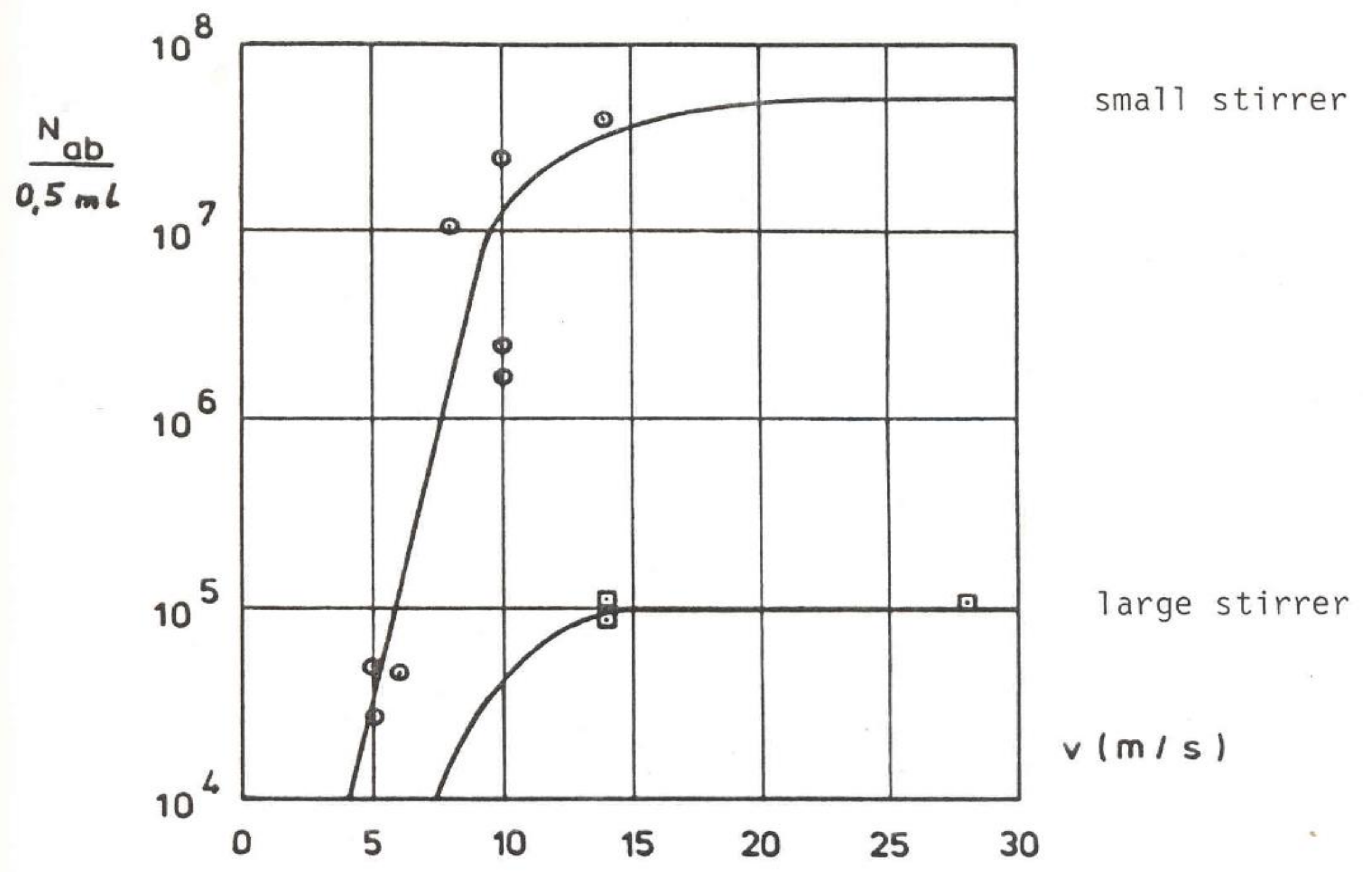
Secondary nucleation in crystallizers



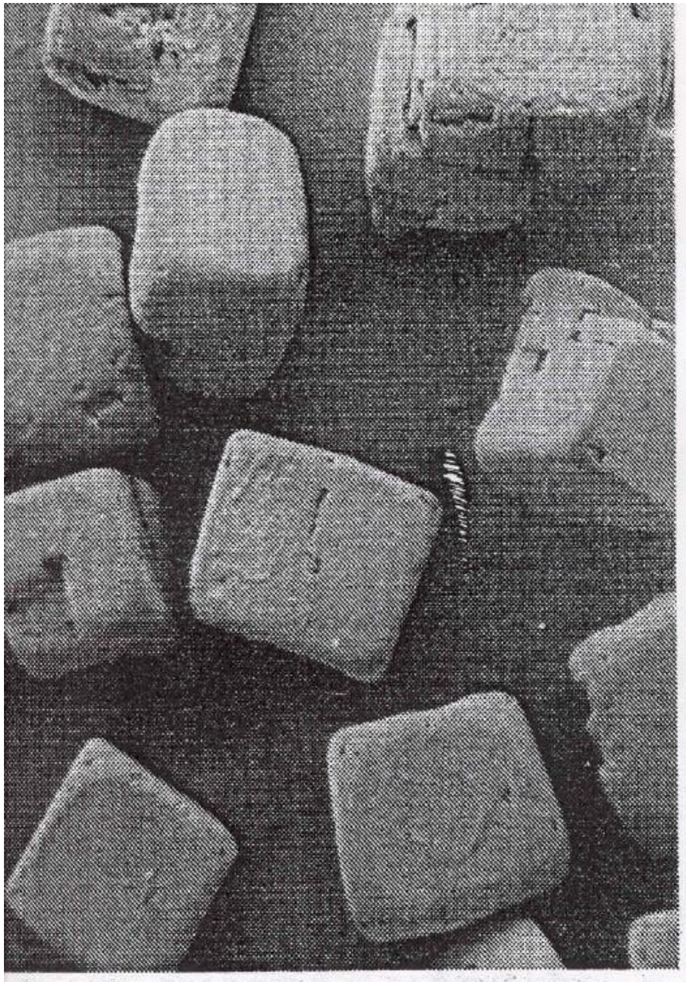
Number of nucleiverses impeller tip speed



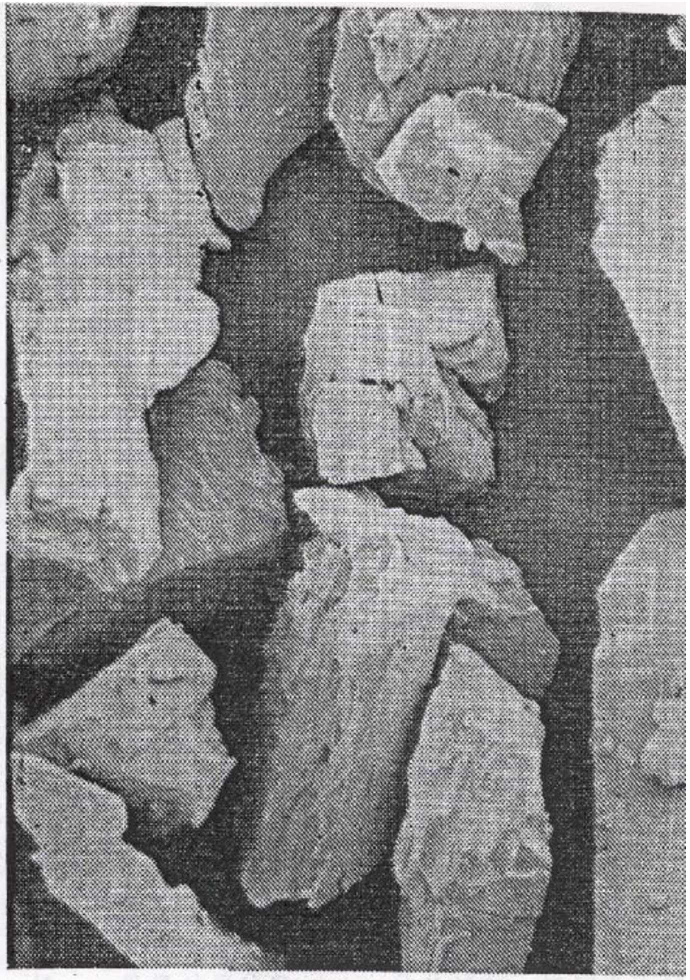
Number of attrition particles depending on stirrer speed for two different size stirrers



Electron microscope photographs (1 mm radial clearance, M = 20 kg/m^3, N = 9 rps, batch time = 120 s)

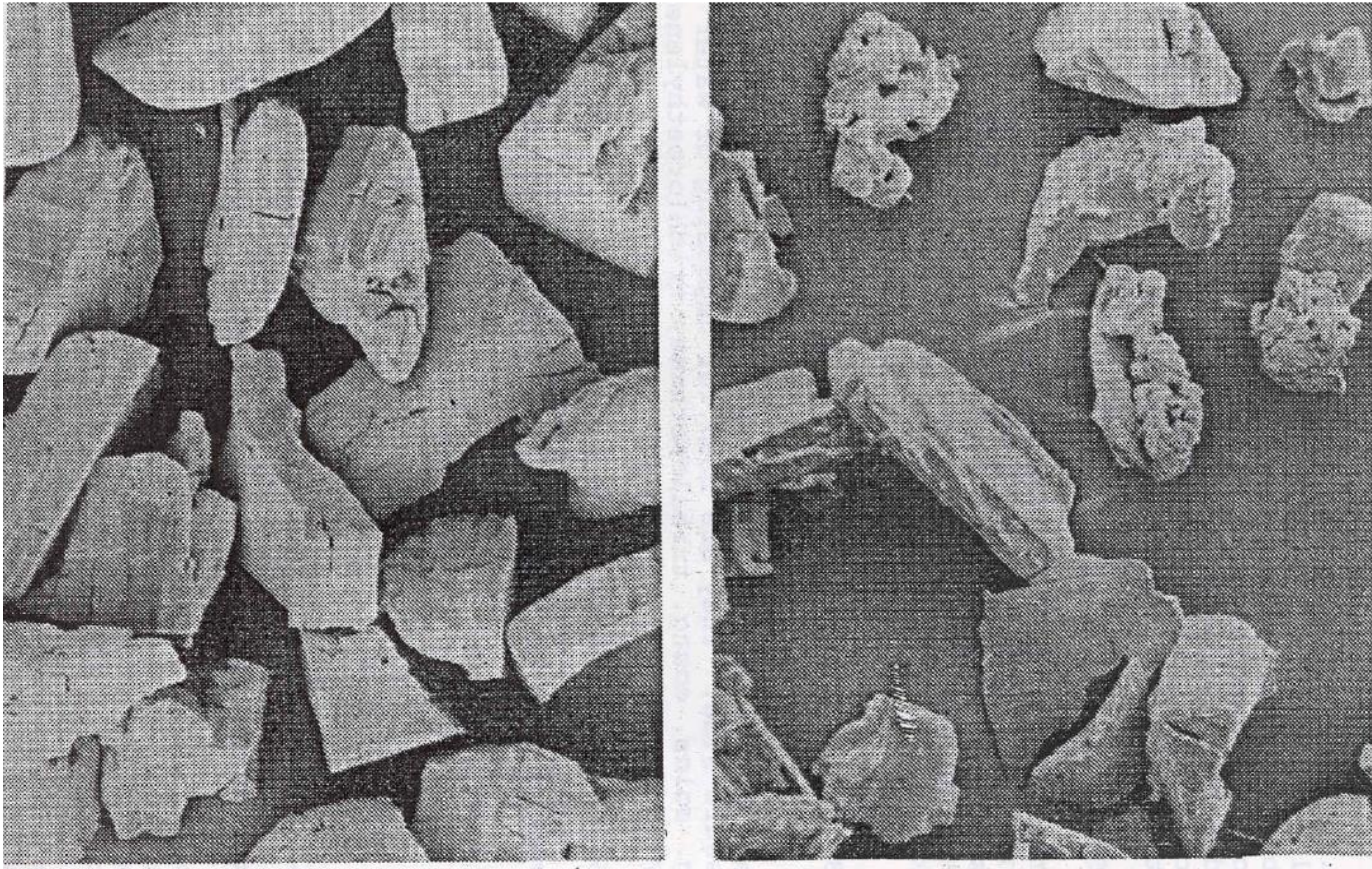


498–592 µm (40 x) Parent crystals



209–249 µm (70 x)

Electron microscope photographs (1 mm radial clearance, M = 20 kg/m^3, N = 9 rps, batch time = 120 s)



88–105 μm (200 x) 62–74 μm (200 x)

Thank you for your attention!

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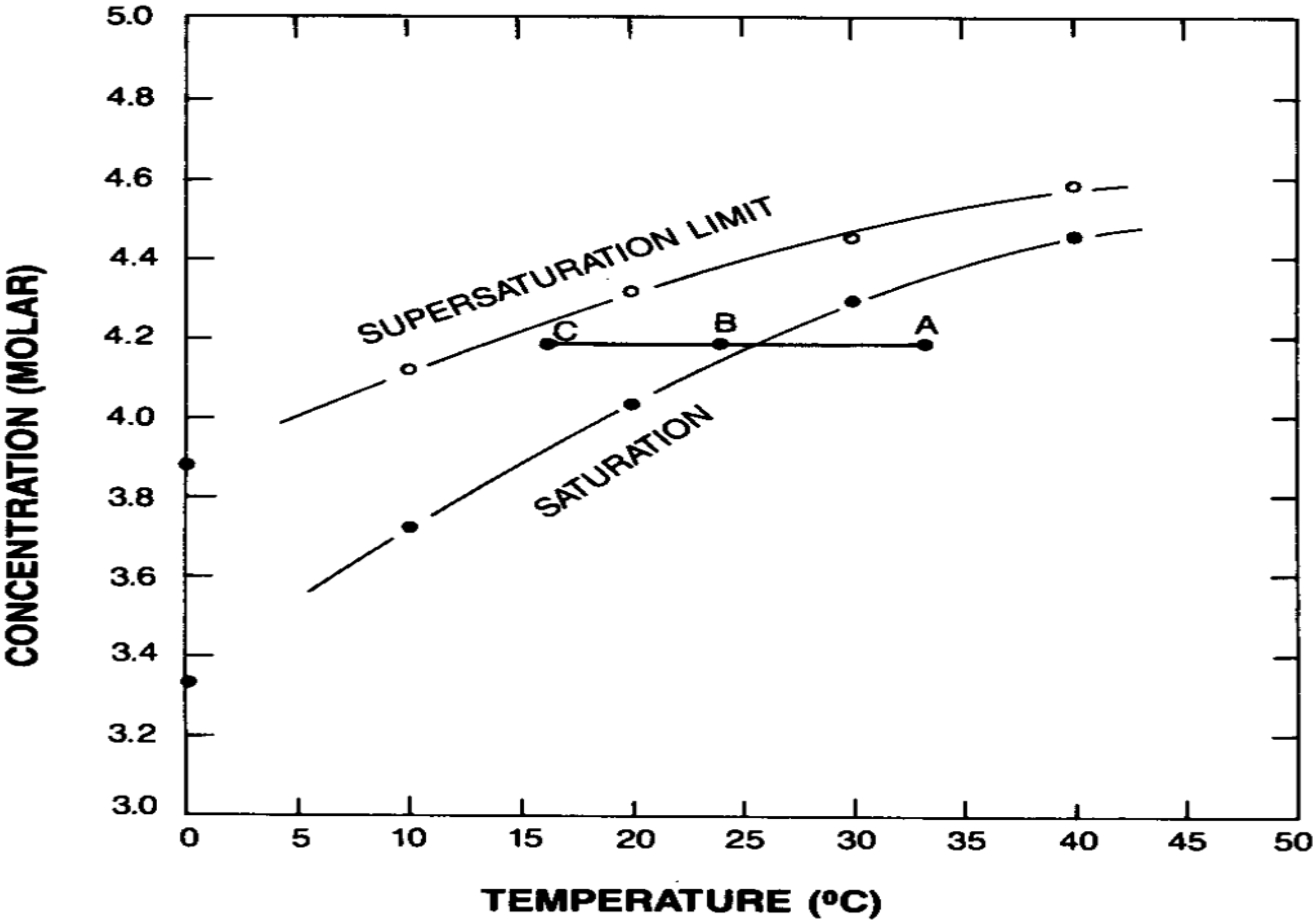
Zentrum für Ingenieurwissenschaften,
Verfahrenstechnik / TVT

Kinetics of crystallization

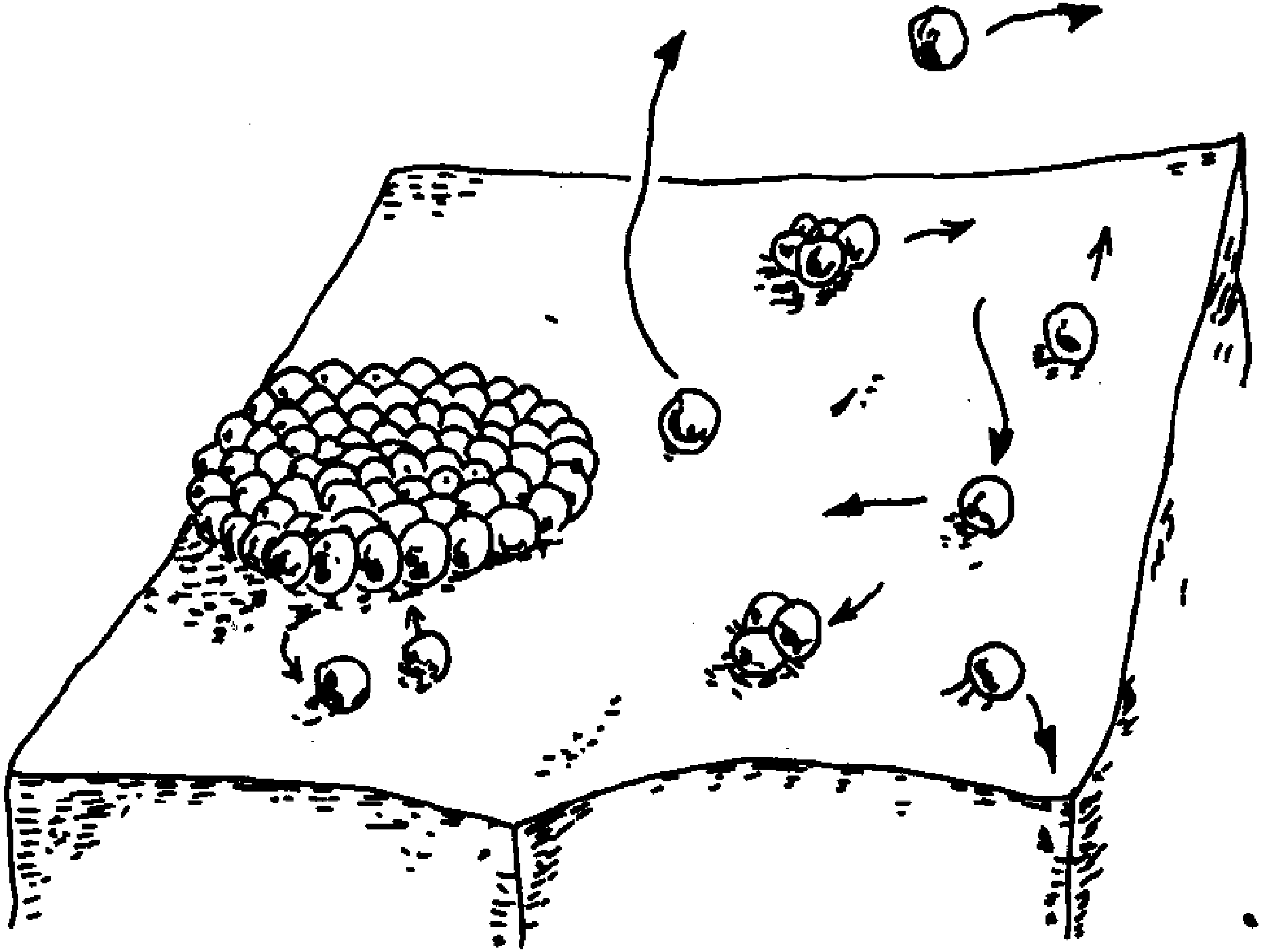
- crystal growth

Prof. Dr.-Ing. Dr. h.c. Joachim Ulrich

Metastable zone width for KCl-water system (Chang 1984)



Formation of two-dimensional critical neucleus on a crystal surface (M. Ohara and R.C. Reid)



Crystal Growth Kinetics

Controlled by Surface Integration: Two-Dimensional Nucleation

General form:

$$R_G = \alpha \sigma^P \exp\left(\frac{\beta}{\sigma}\right)$$

$R_{G,}$	growth rate
α, β, P	constant
σ	relative supersaturation

- Mono-nuclear Model:

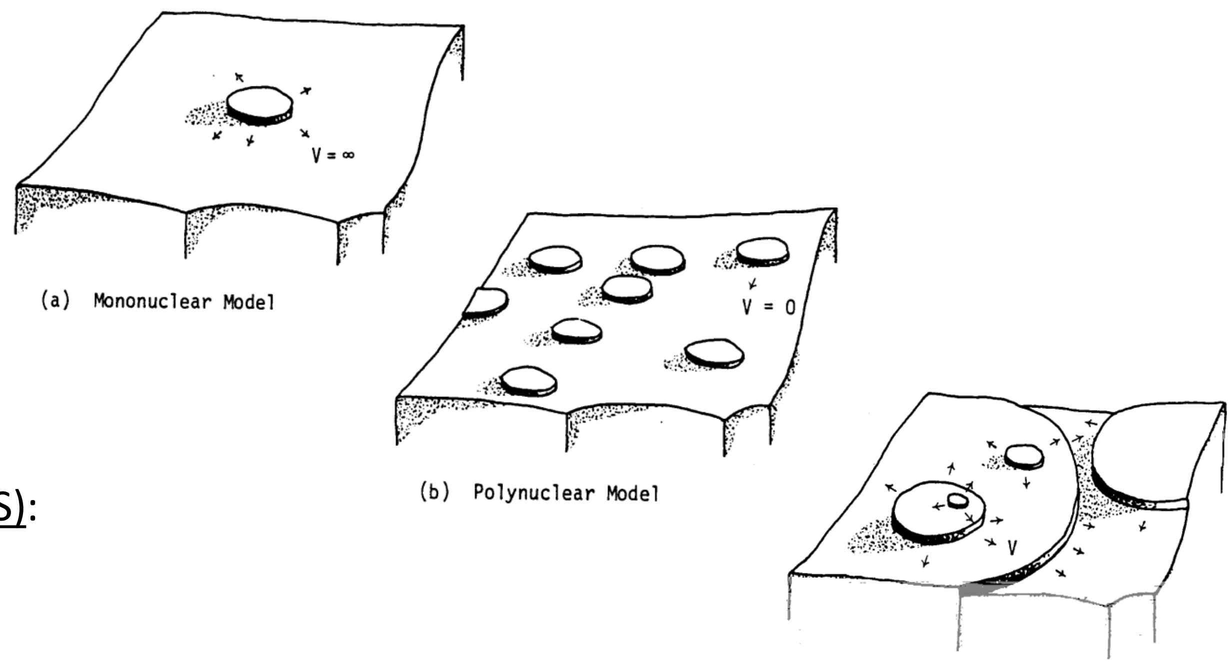
  V= ∞ and P=1/2

- Poly-nuclear Model:

  V=0 and P=3/2

- Birth & Spread Model (B&S):

  V=finite value and P= 5/6



(a) Mononuclear Model

(b) Polynuclear Model

(c) Birth and Spread Model

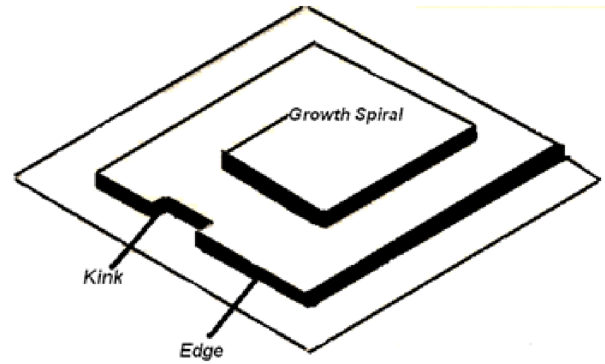
[8] Myerson, A.S., Handbook of Industrial Crystallization; Butterworth-Heinemann; Oxford, UK, 2001, 70-71.

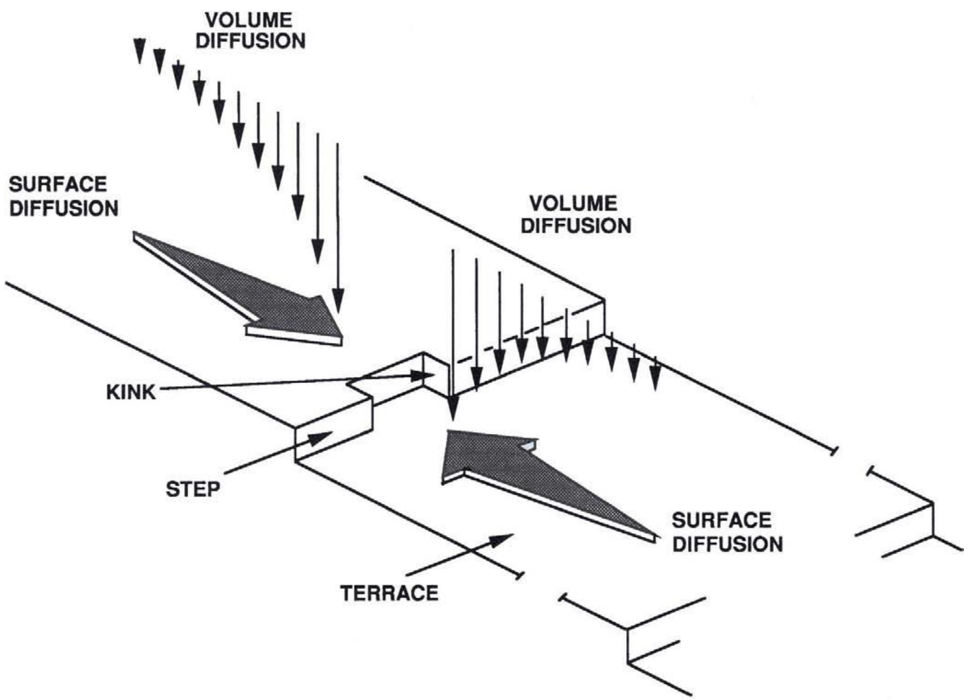
Crystal Growth Kinetics

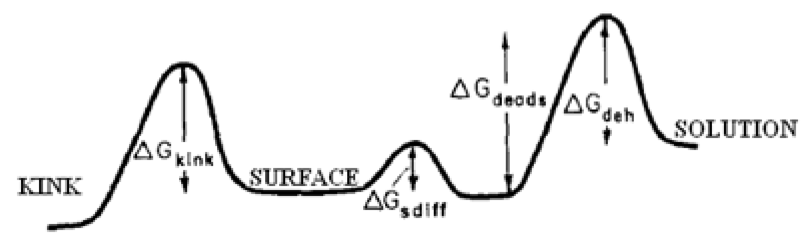
Controlled by Surface Integration: Burton-Cabrera-Frank (BCF) Model

Screw dislocations provide self perpetuating step

- low Supersaturations (BCF): $R_G \propto S^2$
- high Supersaturations (RIG): $R_G \propto S$





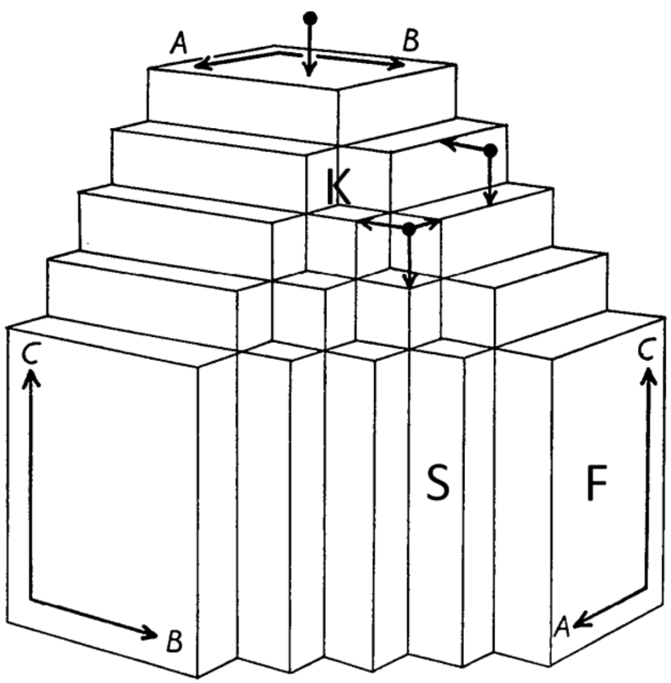


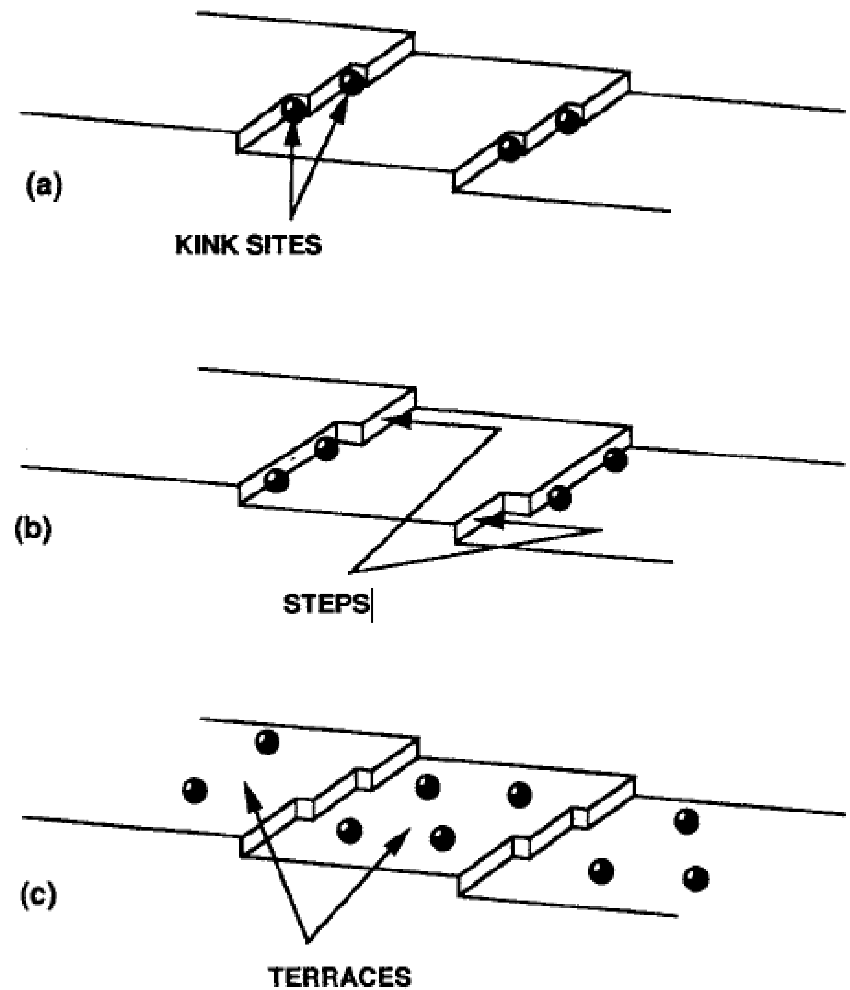
[8] Myerson, A.S., Handbook of Industrial Crystallization; Butterworth-Heinemann; Oxford, UK, 2001 ,70-71.

Crystal Growth Kinetics

Kossel Crystal

$$E_K^{att} \rangle E_S^{att} \rangle E_F^{att}$$





[8] Myerson, A.S., Handbook of Industrial Crystallization; Butterworth-Heinemann; Oxford (2001) 70.
[9] Mullin, J.W., Crystallization; Butterworth Heinemann, Oxford (2001) 271.

Crystal Growth Kinetics

Controlled by Mass Integration: Diffusion Layer Model

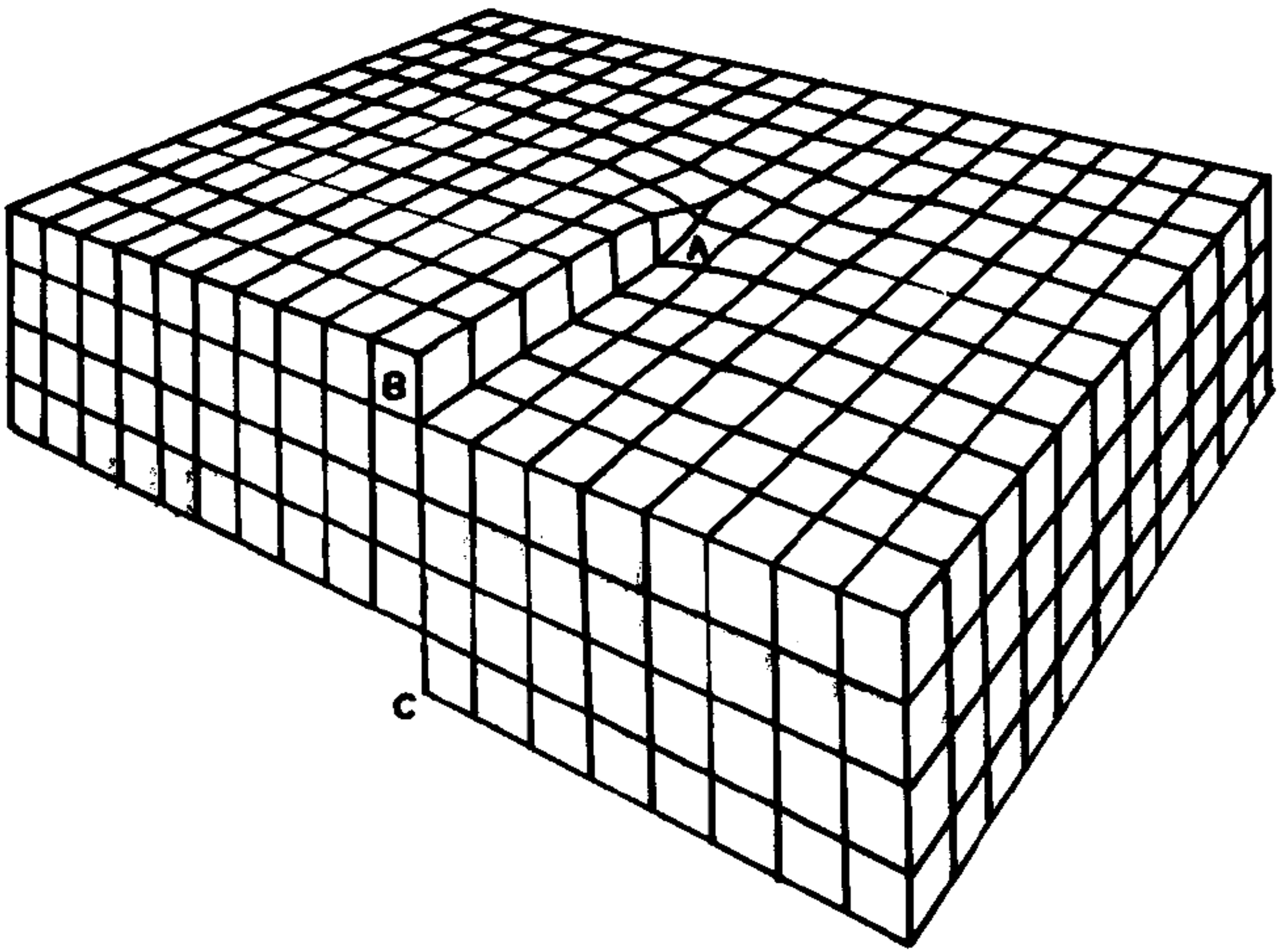
- For one-dimensional case, rate of mass increase equated to diffusion rate through the boundary layer

$$\frac{dm_c}{dt} = DA\left(\frac{dC}{dx}\right) \qquad R_G = \frac{\beta}{3\alpha\rho}\left(\frac{C - C_i}{\delta}\right)D \Rightarrow R_G \propto D$$

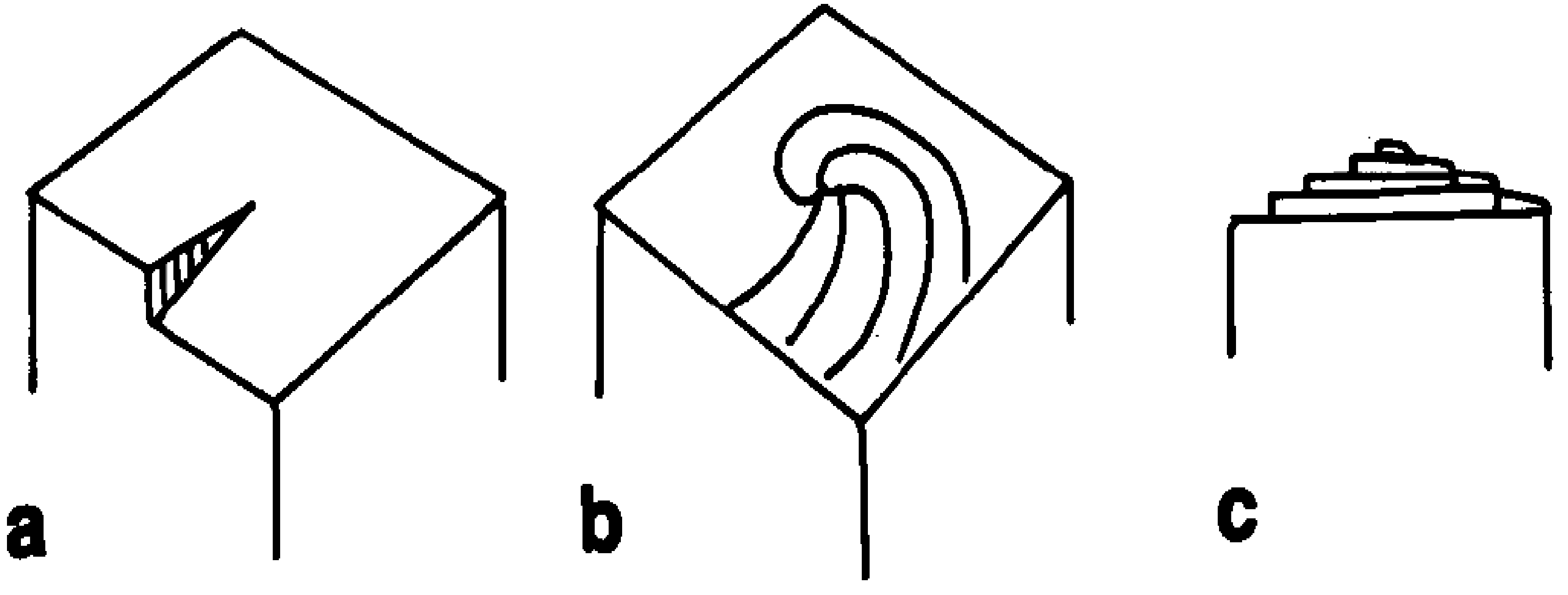
A: surface area of the crystal
D: diffusion coefficient
dC/dx: concentration gradient in boundary layer

α, β: volume and area shape factors
ρ: crystal density
δ: boundary layer thickness
c, c_i: bulk and interfacial concentration

A screw dislocation in a simple cubic crystal.
(Strickland-Constable 1968)



Development of a growth spiral from a screw dislocation
(Mullin 1972)



Definition of growth rates

$$R_G = K_G \, \Delta c^n = \frac{1}{A} \frac{dm}{dt} = \frac{3\alpha}{\beta} \varrho \, G$$

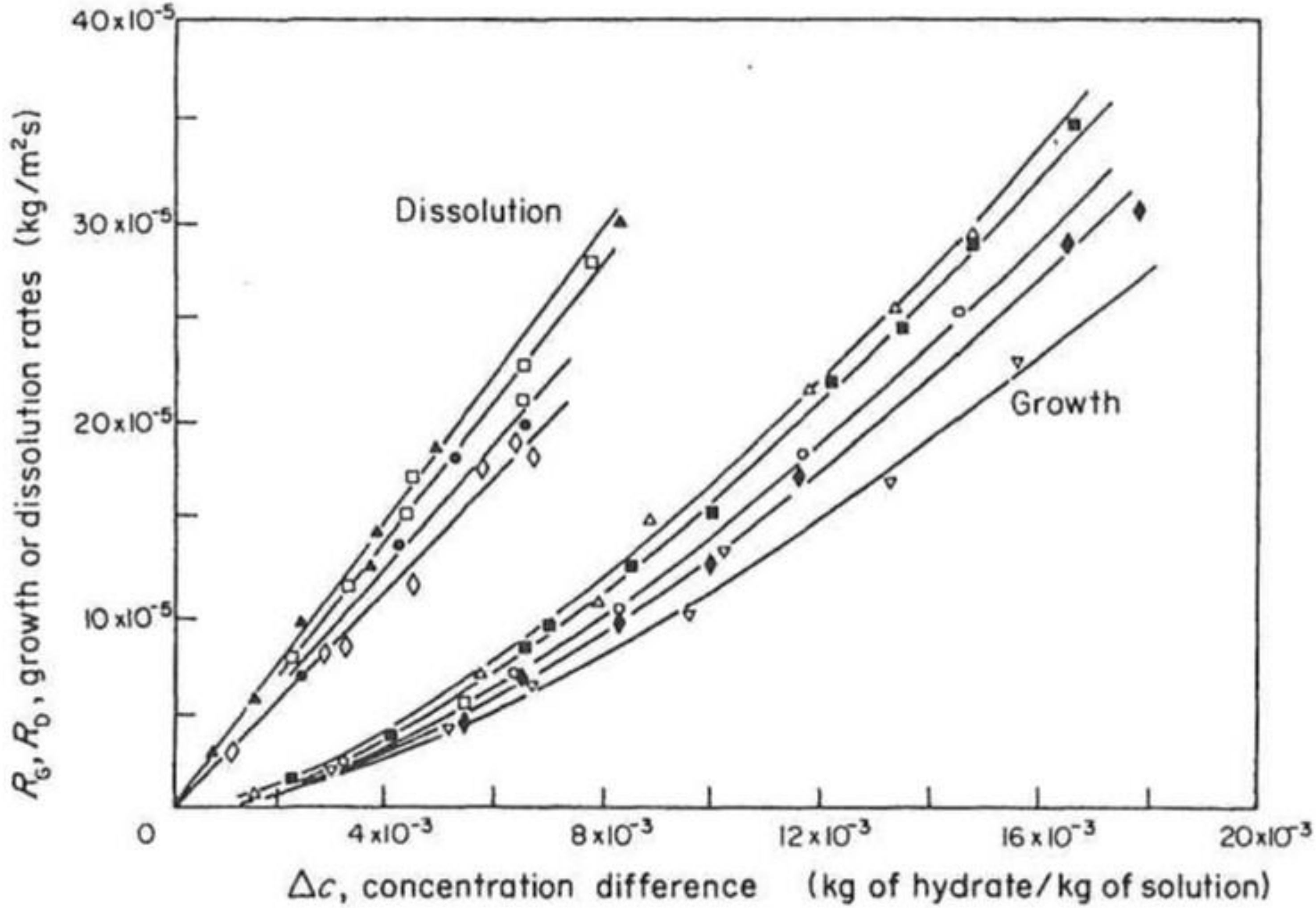
$$[\, kg/m^2 \, s \,]$$ **(mass growth rate)**

with $$G = \frac{dL}{dt} \quad \left[\frac{m}{s} \right]$$ **(linear growth rate)**

with $$m = \alpha \, \varrho \, L^3$$ **(mass)**

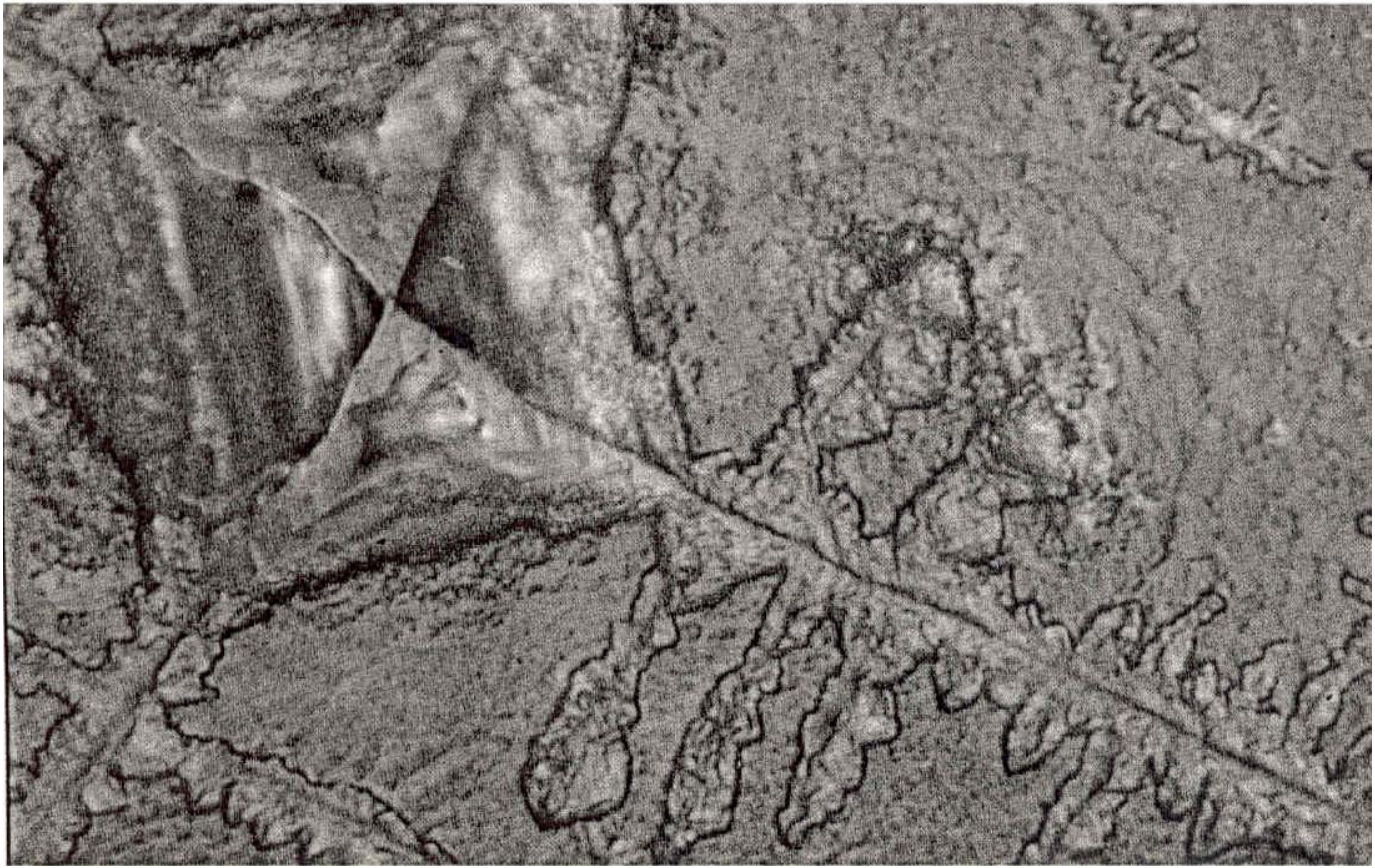
with $$A = \beta \, L^2$$ **(area)**

$$R_G = a \, L^{-m} \, \Delta c^n$$ **(modeled growth rate)**



40 x 10^-5
30 x 10^-5
20 x 10^-5
10 x 10^-5
0
4 x 10^-3
8 x 10^-3
12 x 10^-3
16 x 10^-3
20 x 10^-3
Dissolution
Growth
R_G, R_D, growth or dissolution rates (kg/m²s)
Δc, concentration difference (kg of hydrate/kg of solution)

A container of human interferon crystals

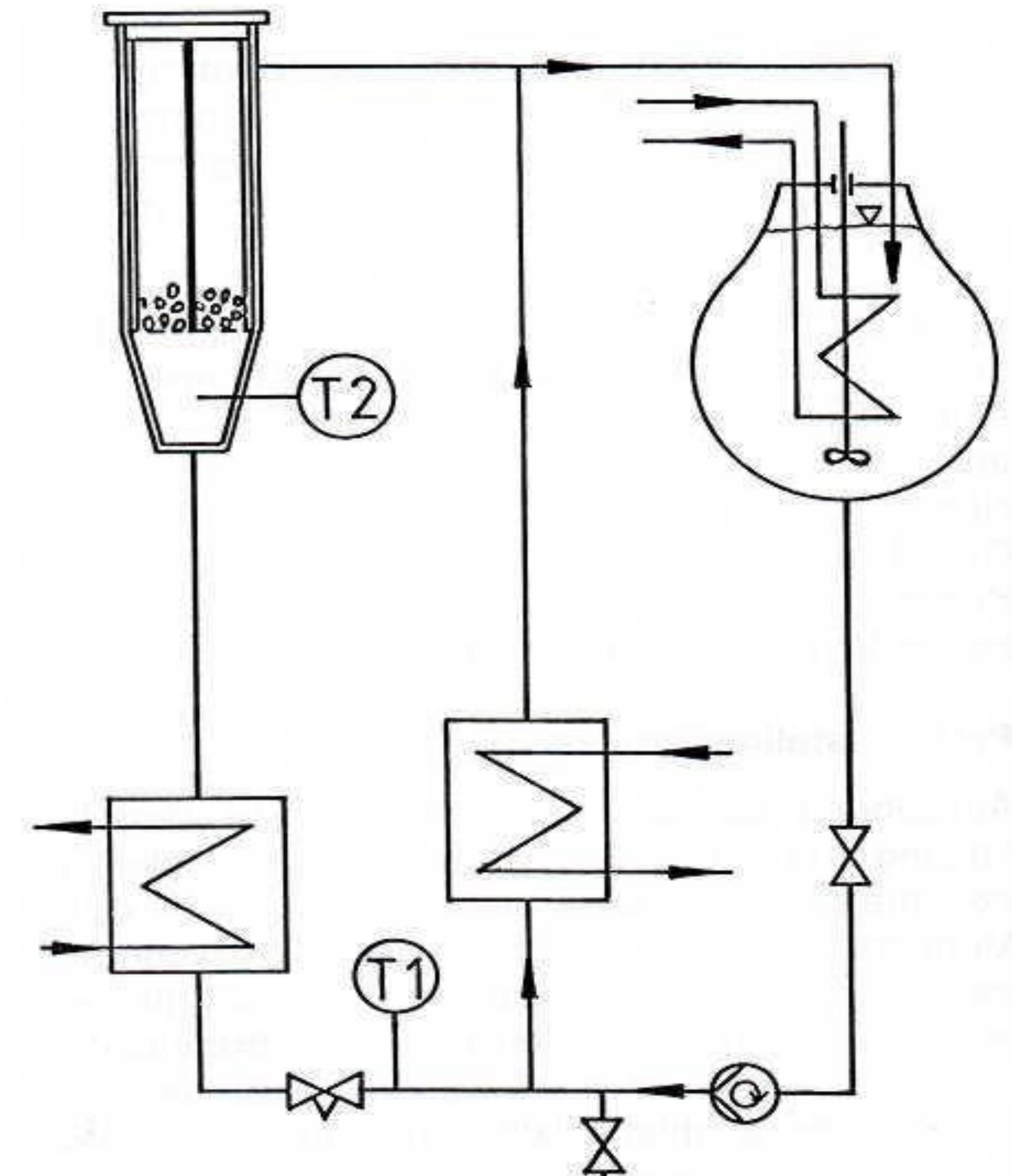


Photomicrograph of human interferon, magnification x 160

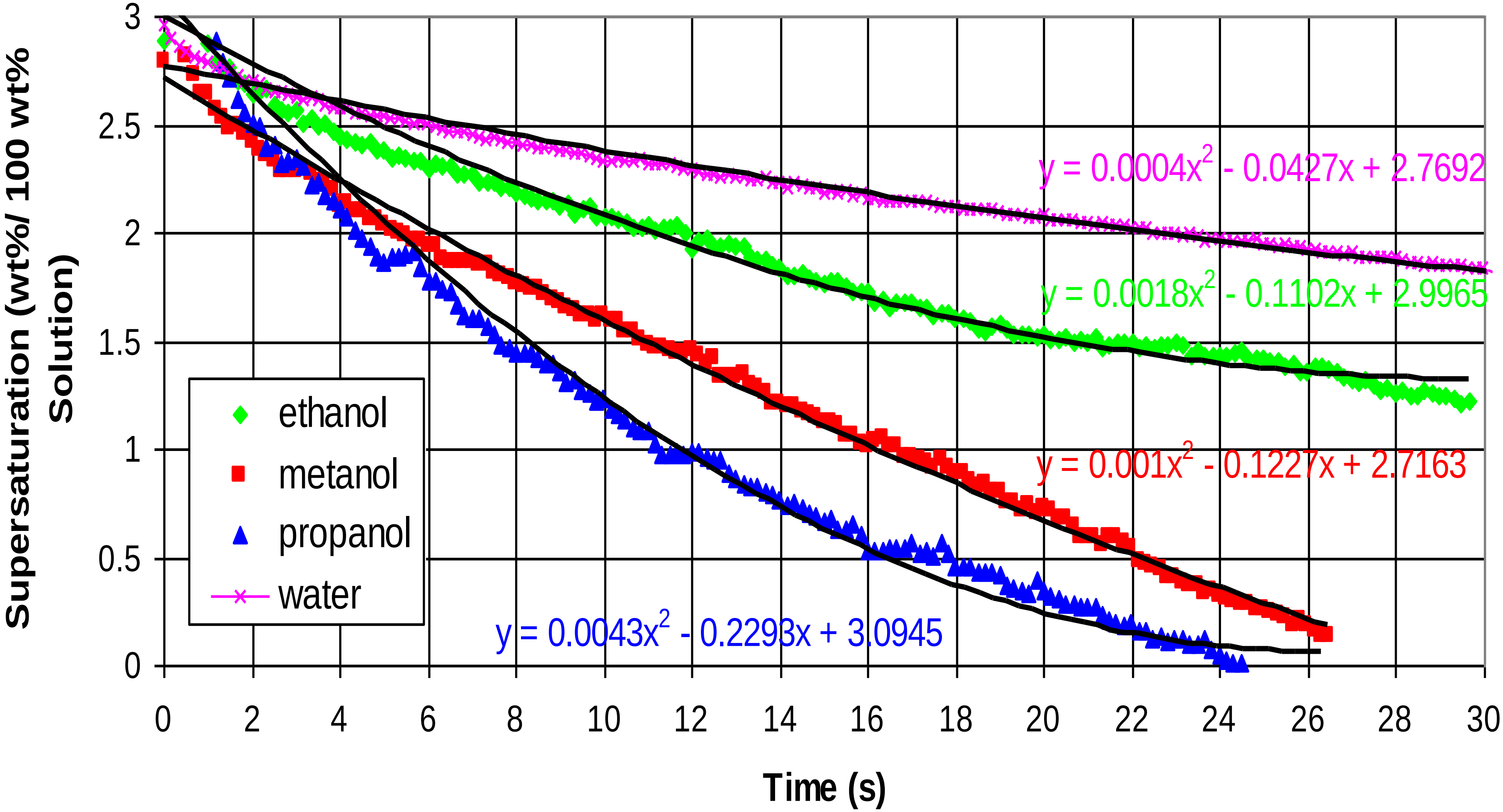
Influence on the crystal growth rate

- supersaturation
- temperature level
- fluiddynamic constitutions
- (crystal size)
- crystal surface quality
- history of crystals (growth time)
- impurities, additives

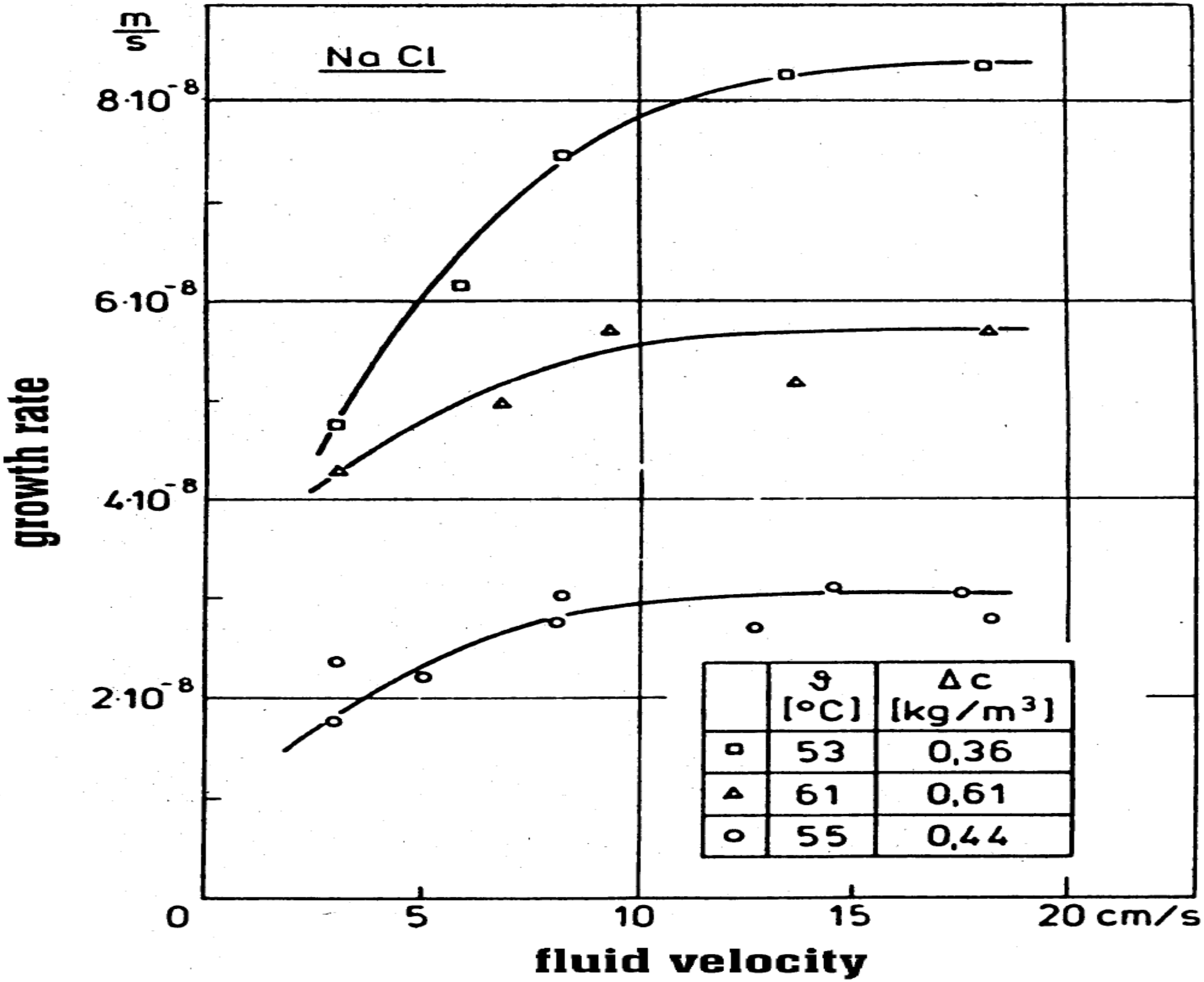
Fluidized bed crystallizer



Growth rates of NaCl crystals in a the fluidized

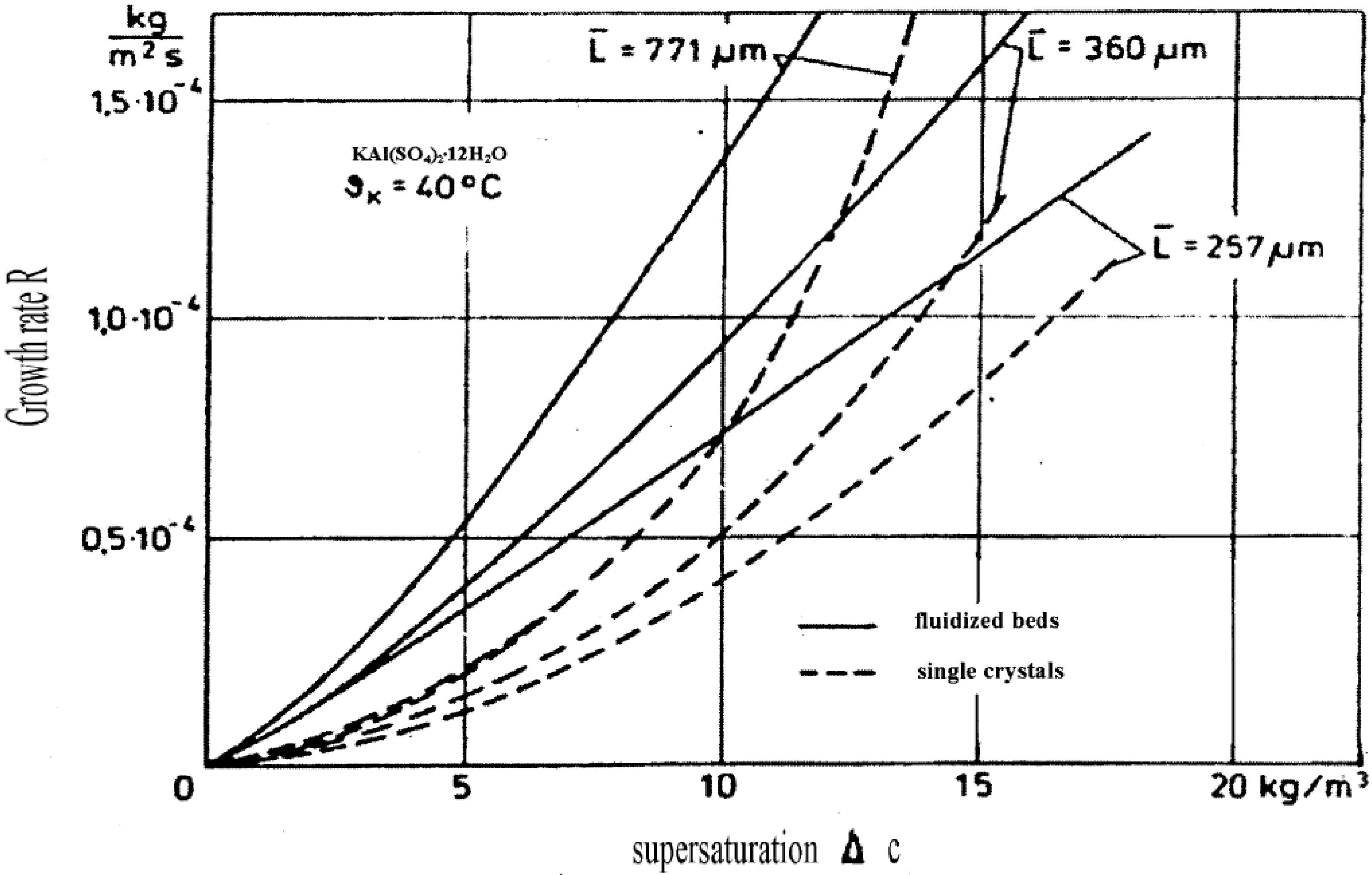


Influence of fluid velocity on locally fixed NaCl crystals

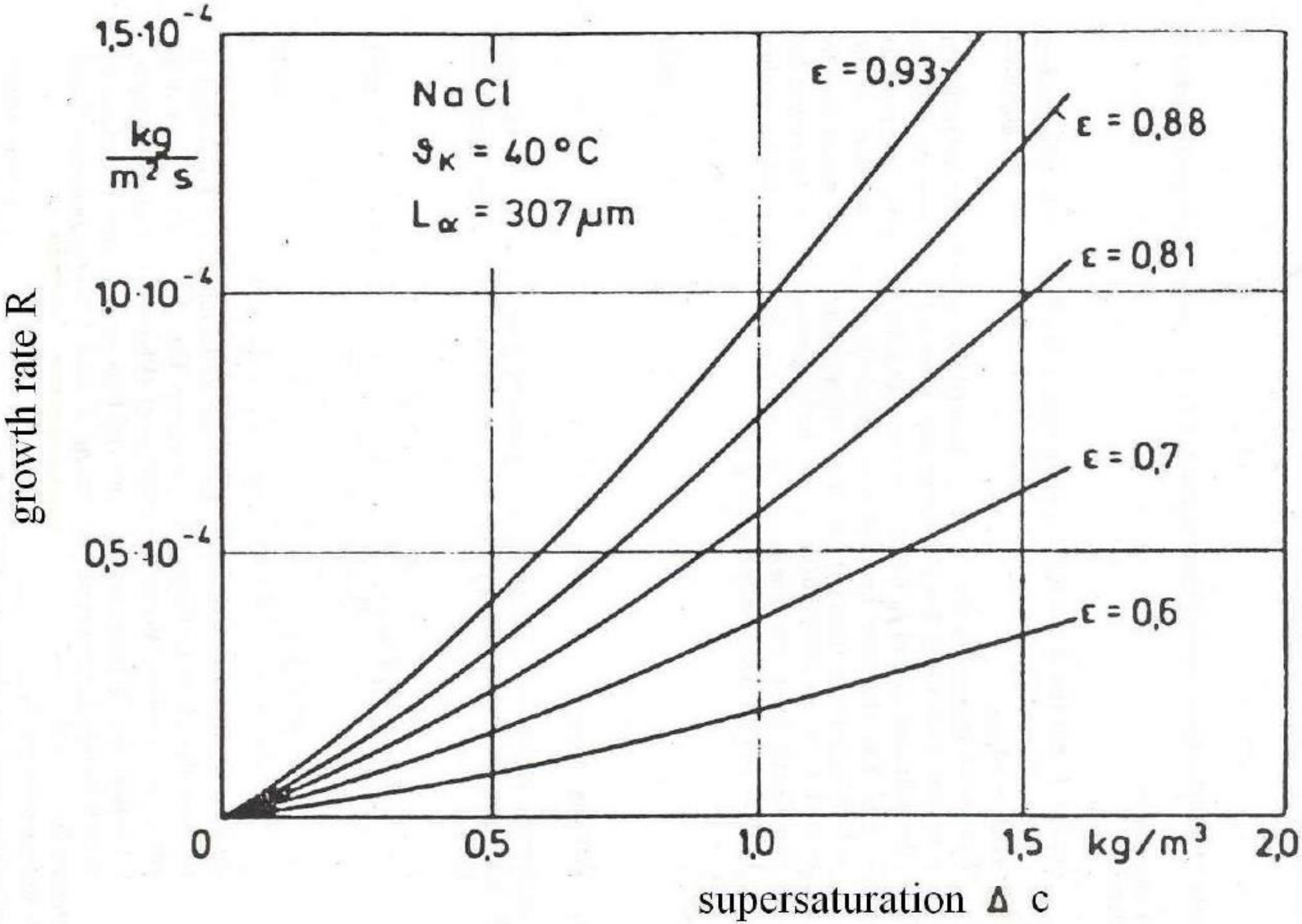


Growth rates: Comparison of single free suspended crystals and crystals grown in an fluidized bed

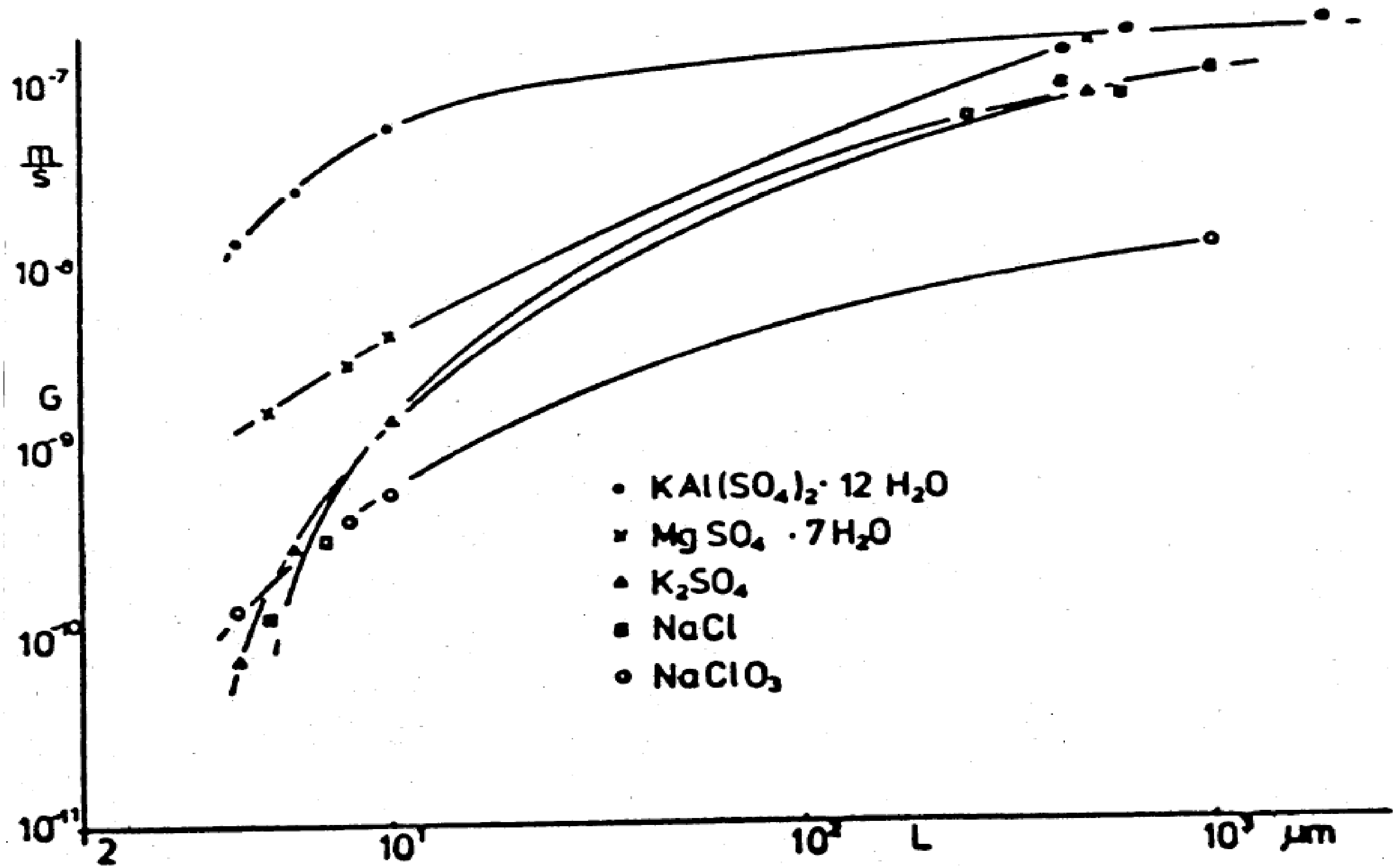
1



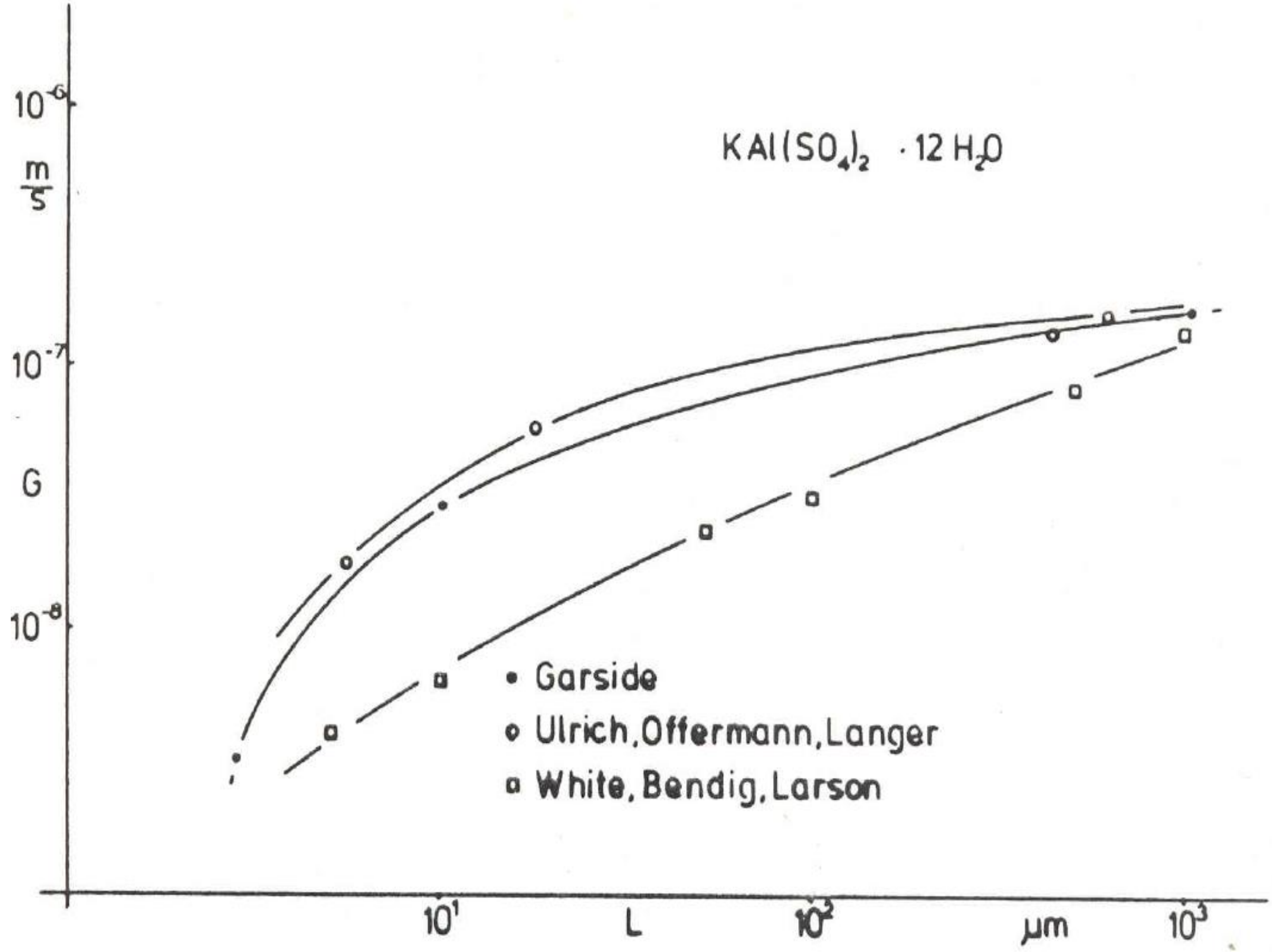
Growth rate of NaCl crystals in a fluidized bed, here: Influence of suspension density ε



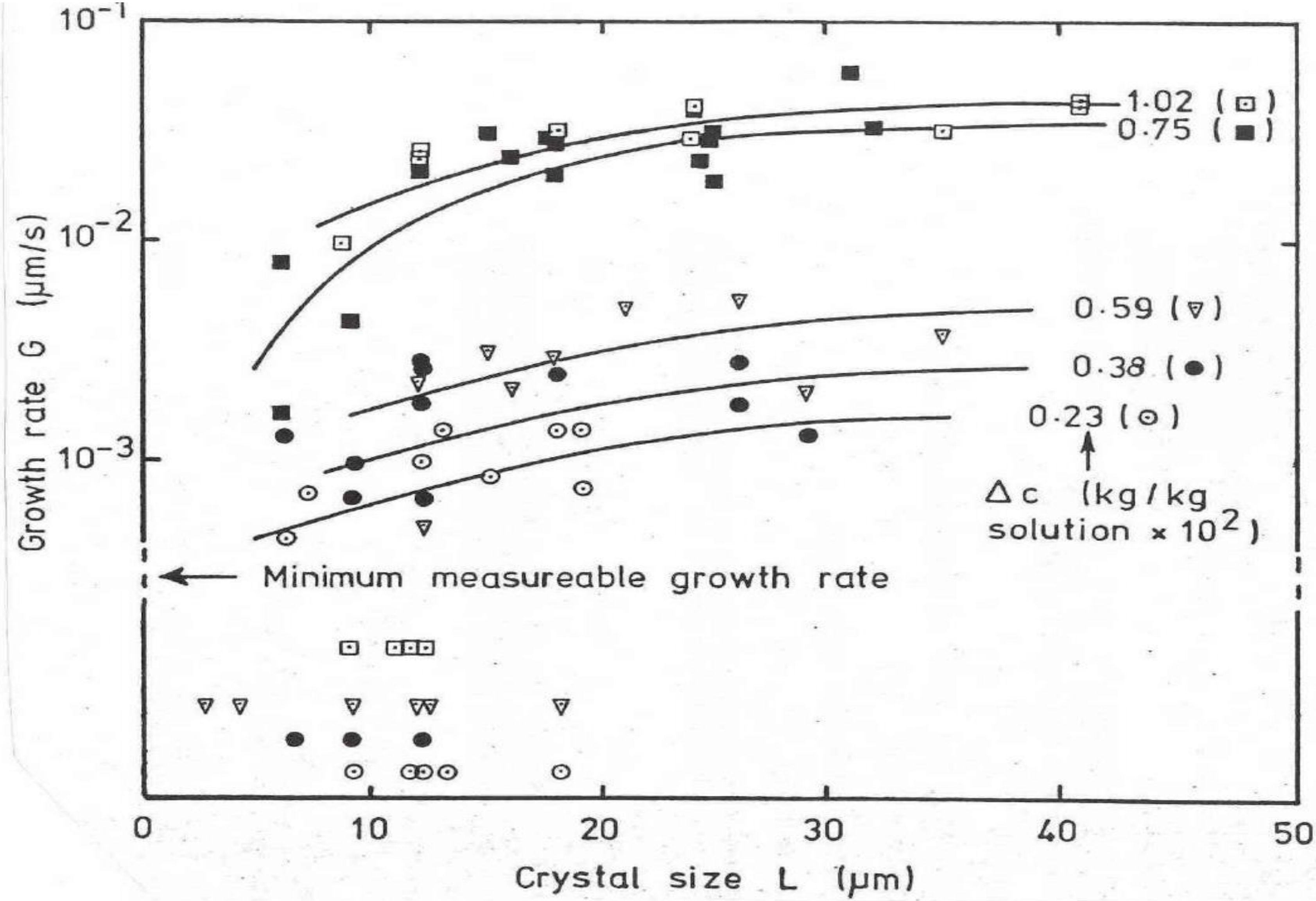
Growth rates of small and product size crystals



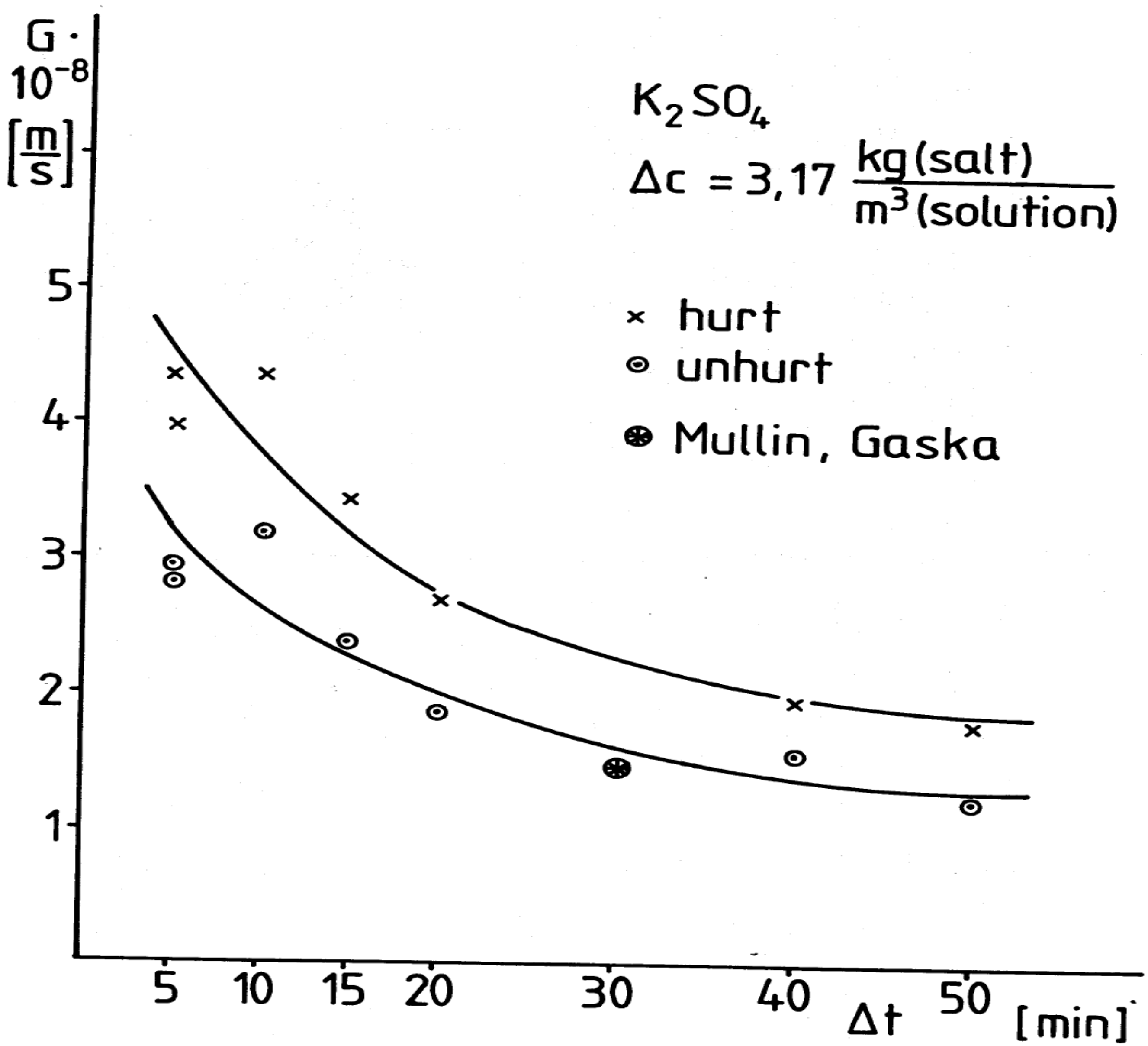
$KAl(SO_4)_2 \cdot 12H_2O$ growth rates



Growth rates $KAl(SO_4)_2 \cdot 12H_2O$ (Garside 1978)

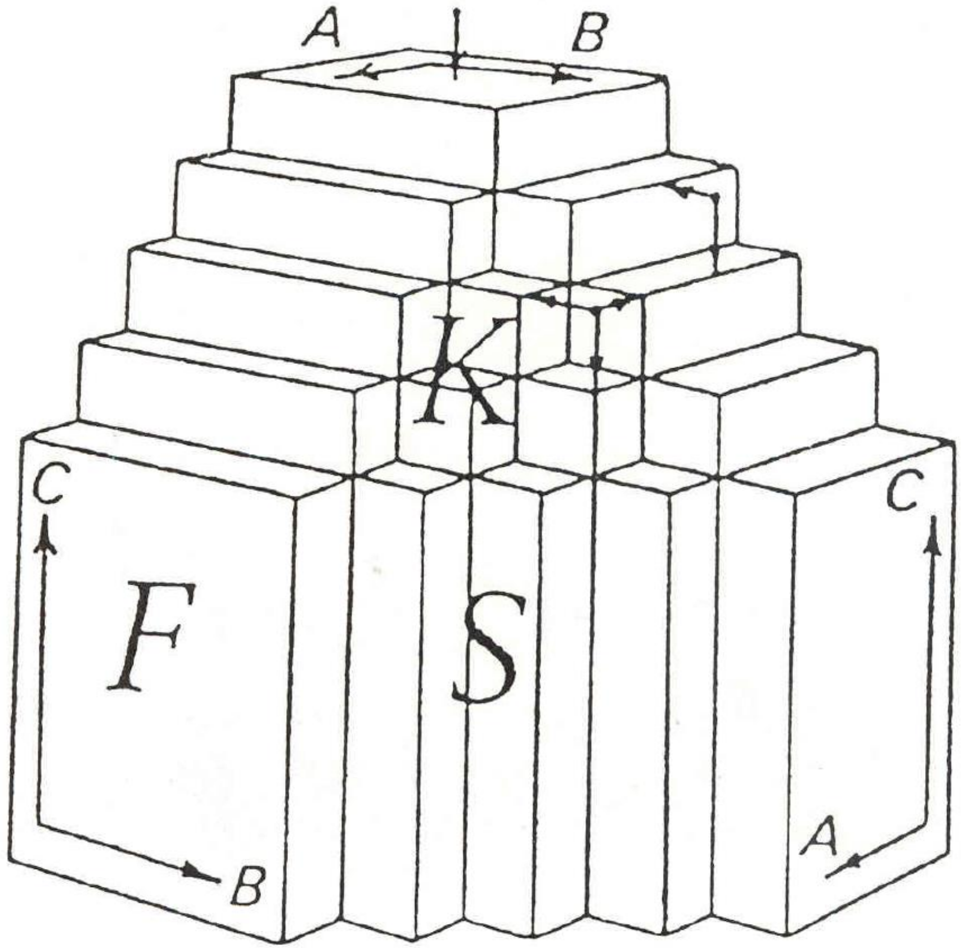


Different growth rates of crystals with different surface qualities in respect with time

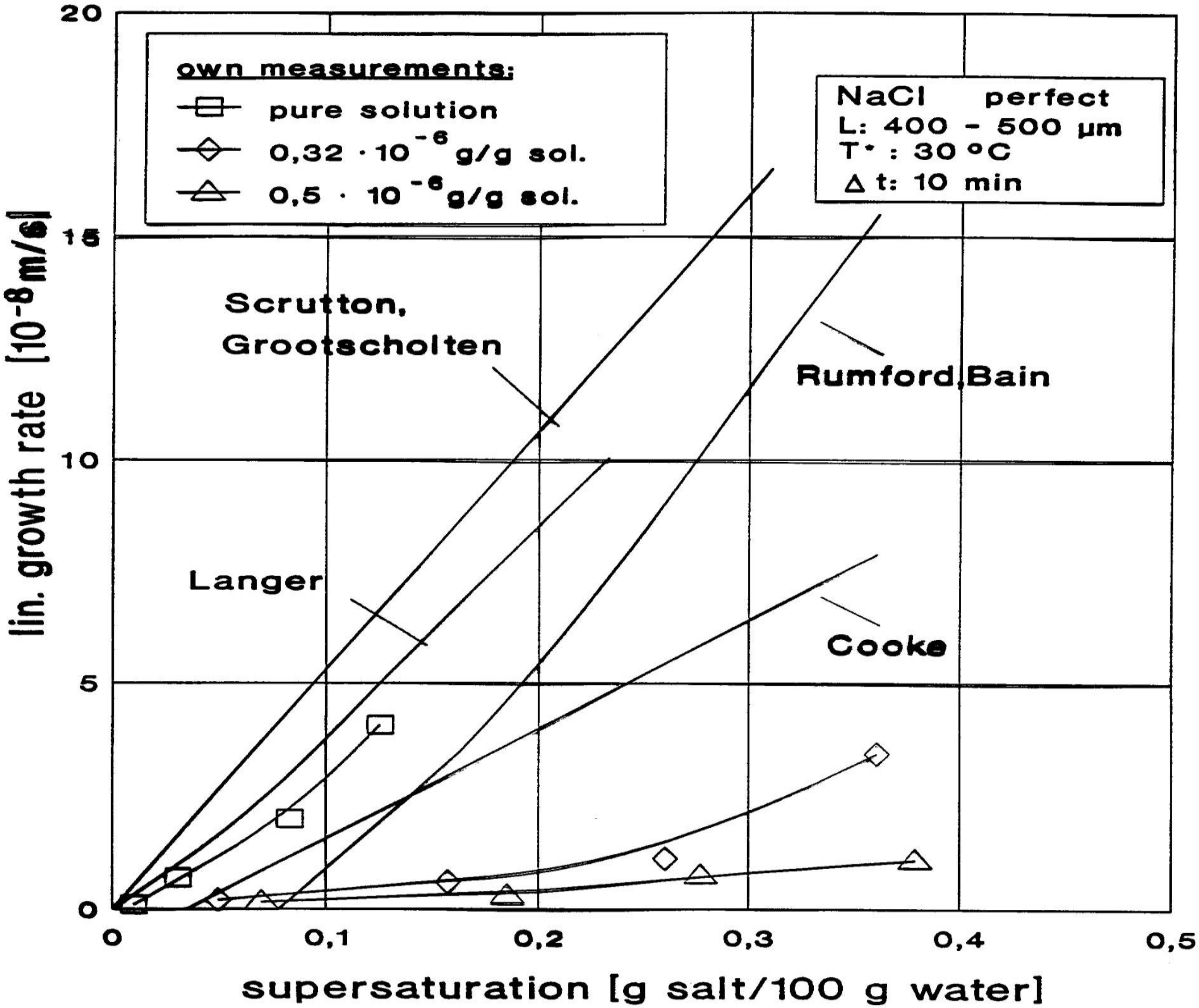


Hypothetical crystal with three PBC's: A // [100], B // [010] and C // [001]. Flat faces are: (100), (010) and (001). Stepped faces are: (110), (101) and (011). Kinked face is (111).

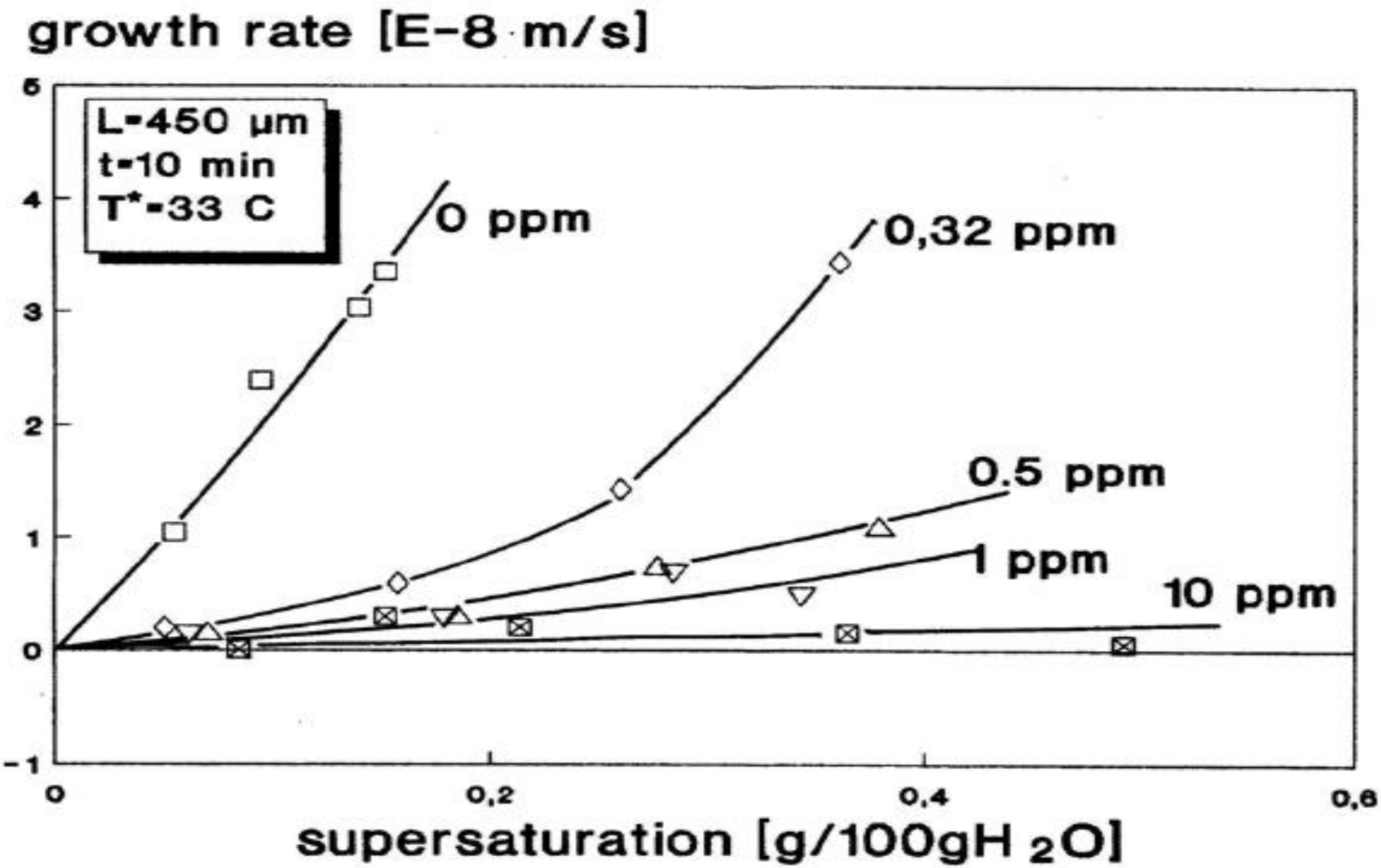
P. Hartman, Structure and morphology



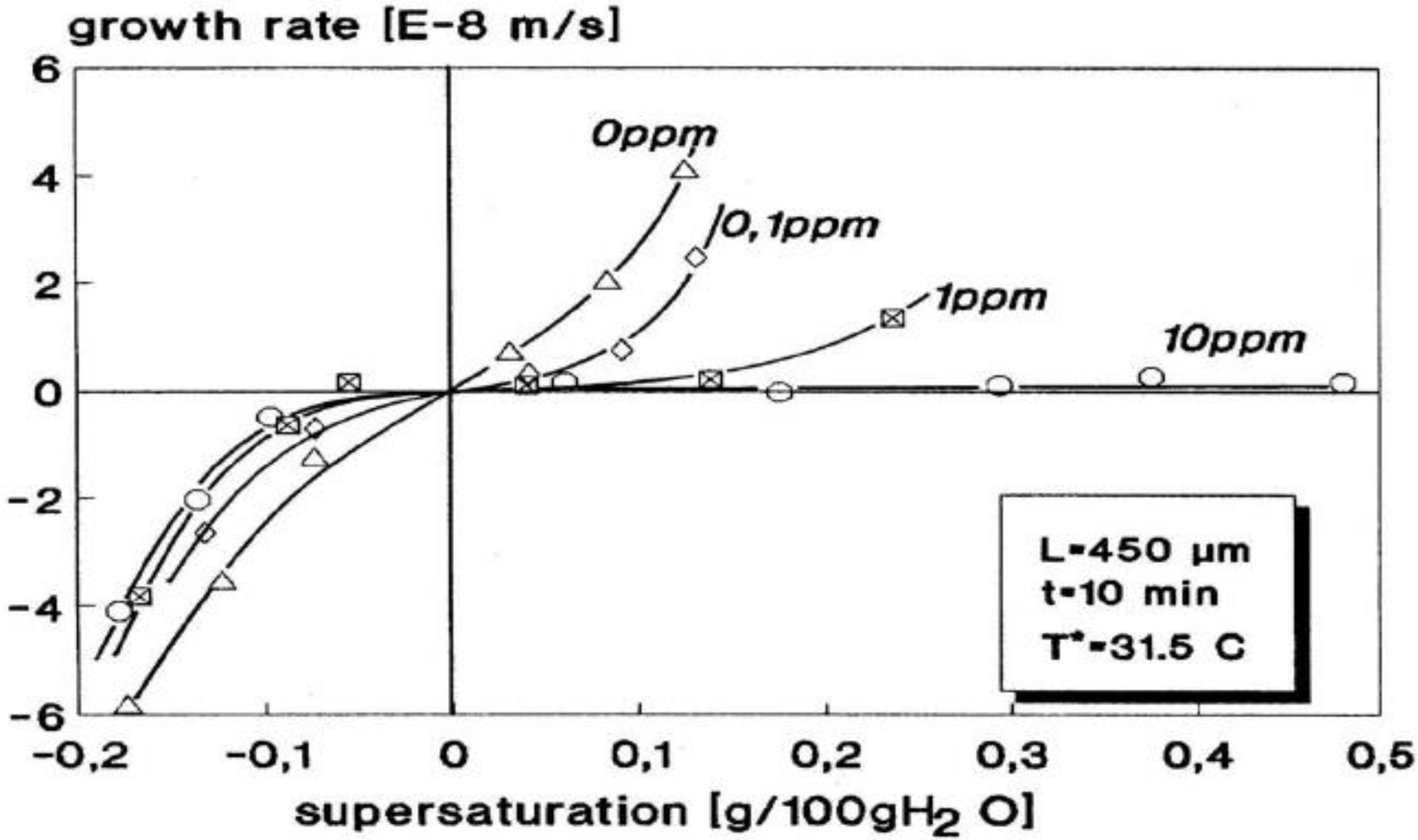
Growth rates of NaCl crystals according to literature

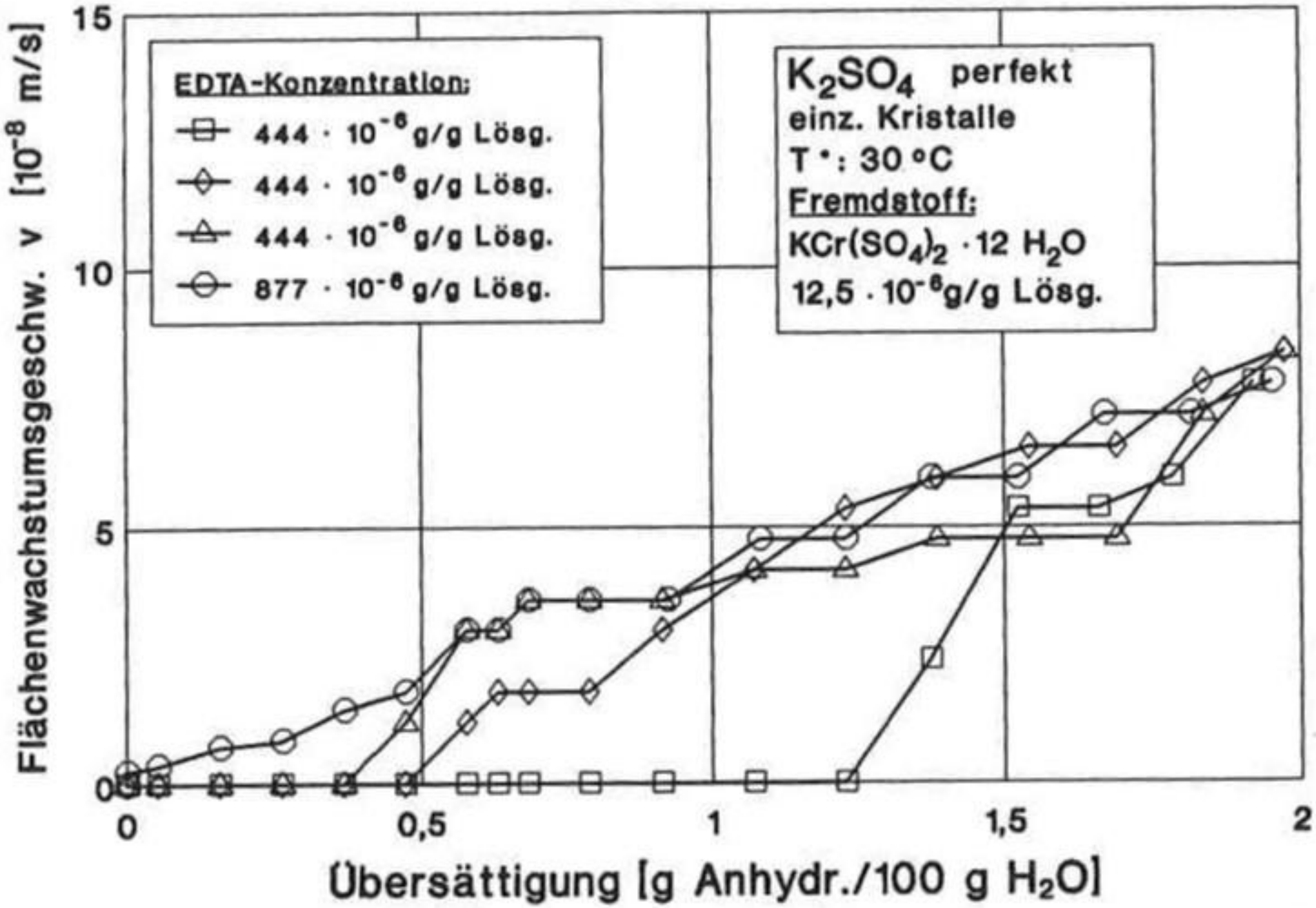


Growth rates of NaCl crystals in the presence of $PbCl_2$

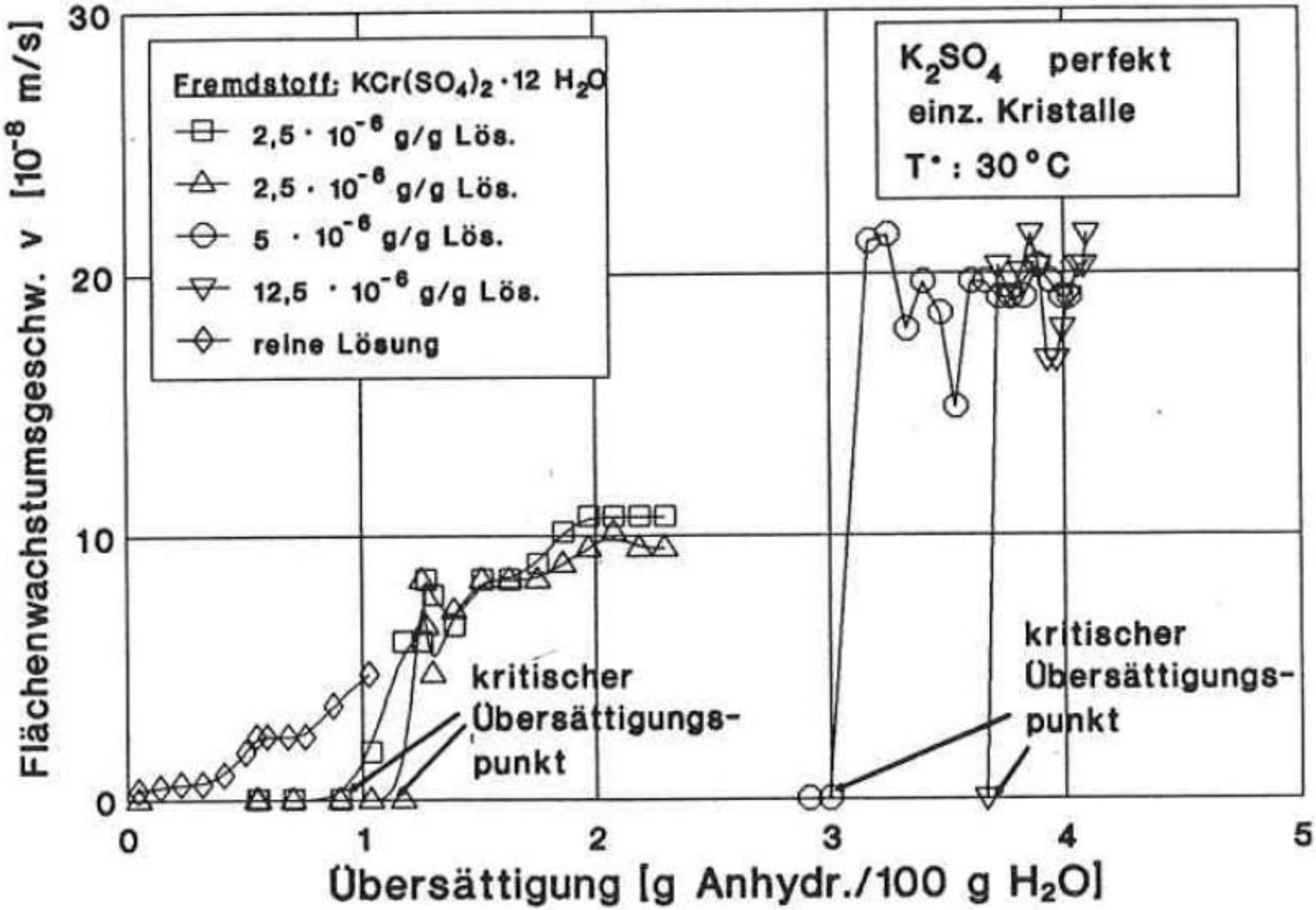


Growth rates of NaCl crystals in the presence of $K_3Fe(CN)_6$



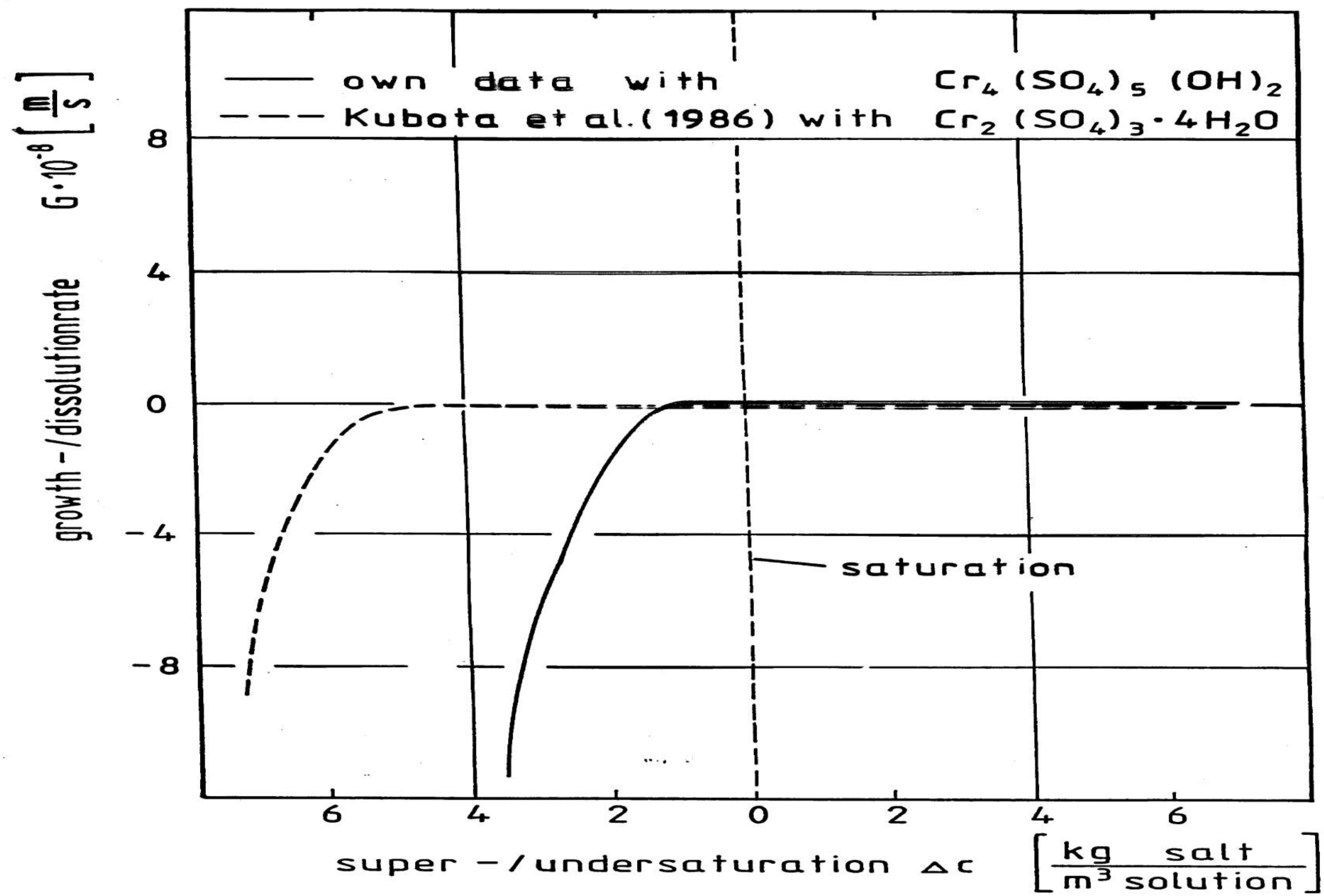


EDTA-Konzentration:
444 · 10^-6 g/g Lösg.
444 · 10^-6 g/g Lösg.
444 · 10^-6 g/g Lösg.
877 · 10^-6 g/g Lösg.
K2SO4 perfekt
einz. Kristalle
T*: 30 °C
Fremdstoff:
KCr(SO4)2 · 12 H2O
12,5 · 10^-6 g/g Lösg.
Flächenwachstumsgeschw. v [10^-8 m/s]
Übersättigung [g Anhydr./100 g H2O]
0
5
10
15
0
0,5
1
1,5
2

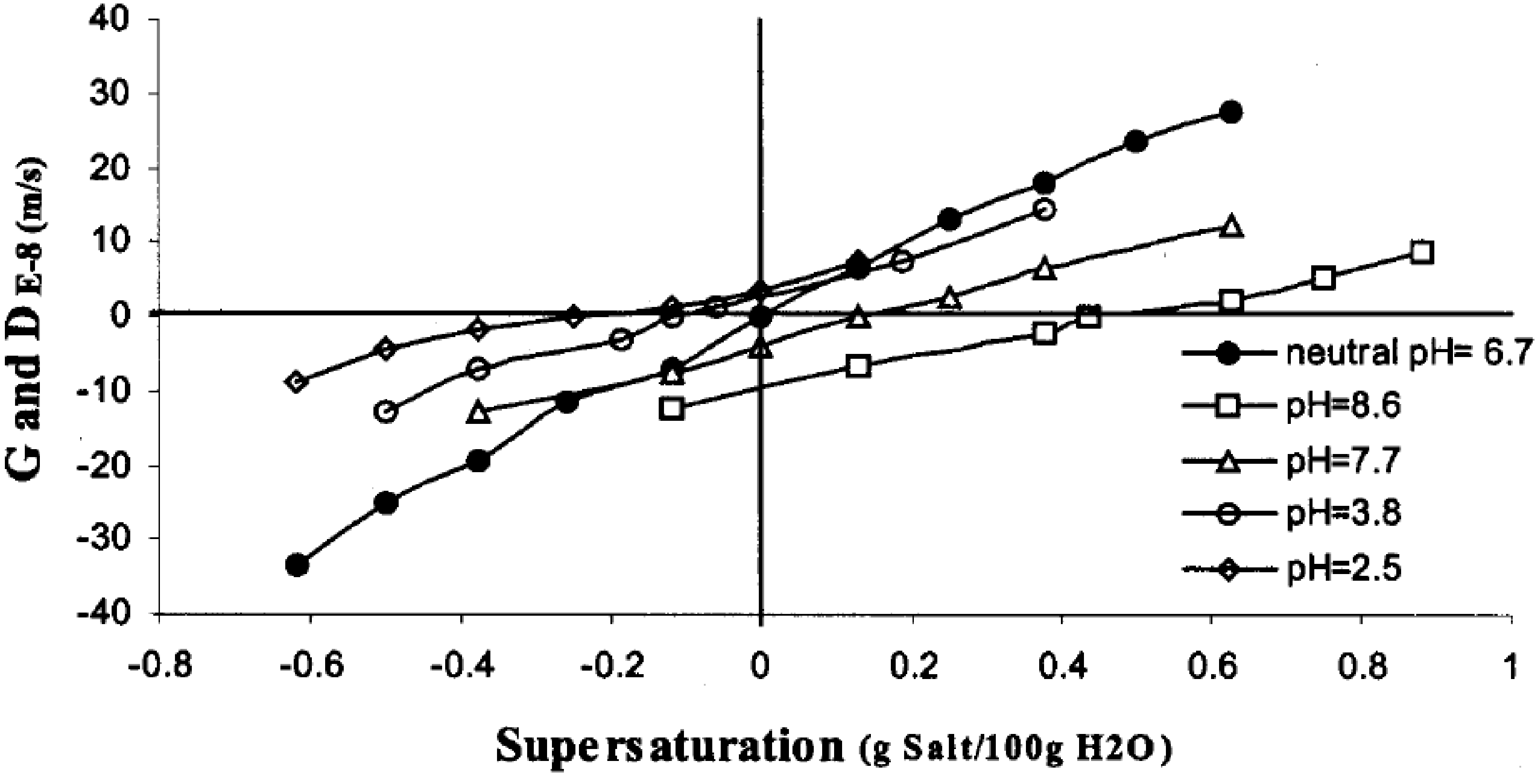


Fremdstoff: KCr(SO4)2 ·12 H2O
2,5 · 10^-6 g/g Lös.
2,5 · 10^-6 g/g Lös.
5 · 10^-6 g/g Lös.
12,5 · 10^-6 g/g Lös.
reine Lösung
K2SO4 perfekt
einz. Kristalle
T* : 30 °C
kritischer Übersättigungs-punkt
kritischer Übersättigungs-punkt
Flächenwachstumsgeschw. v [10^-8 m/s]
Übersättigung [g Anhydr./100 g H2O]
0
10
20
30
0
1
2
3
4
5

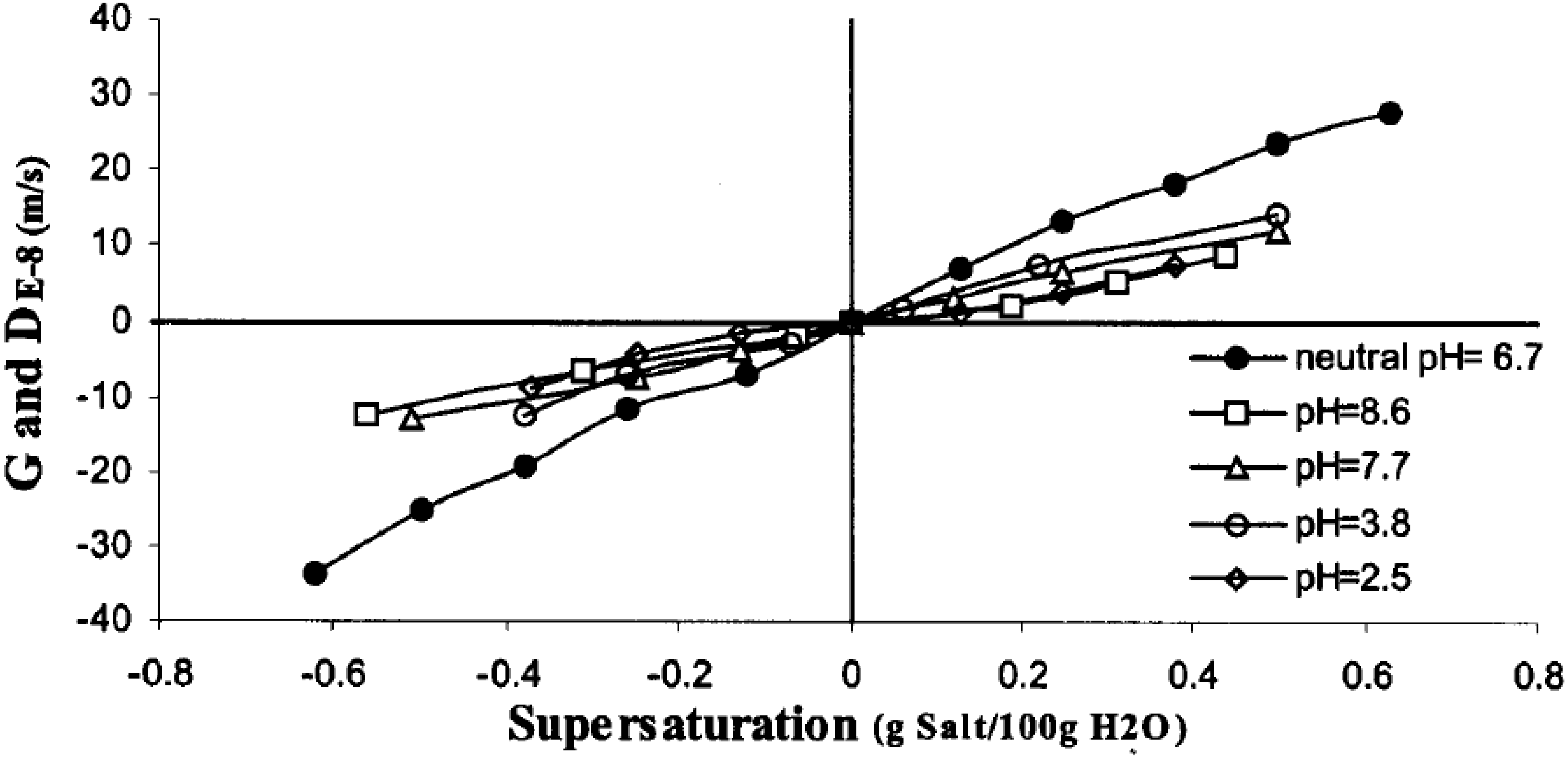
Suppressed dissolution rates of K_2SO_4 by additives



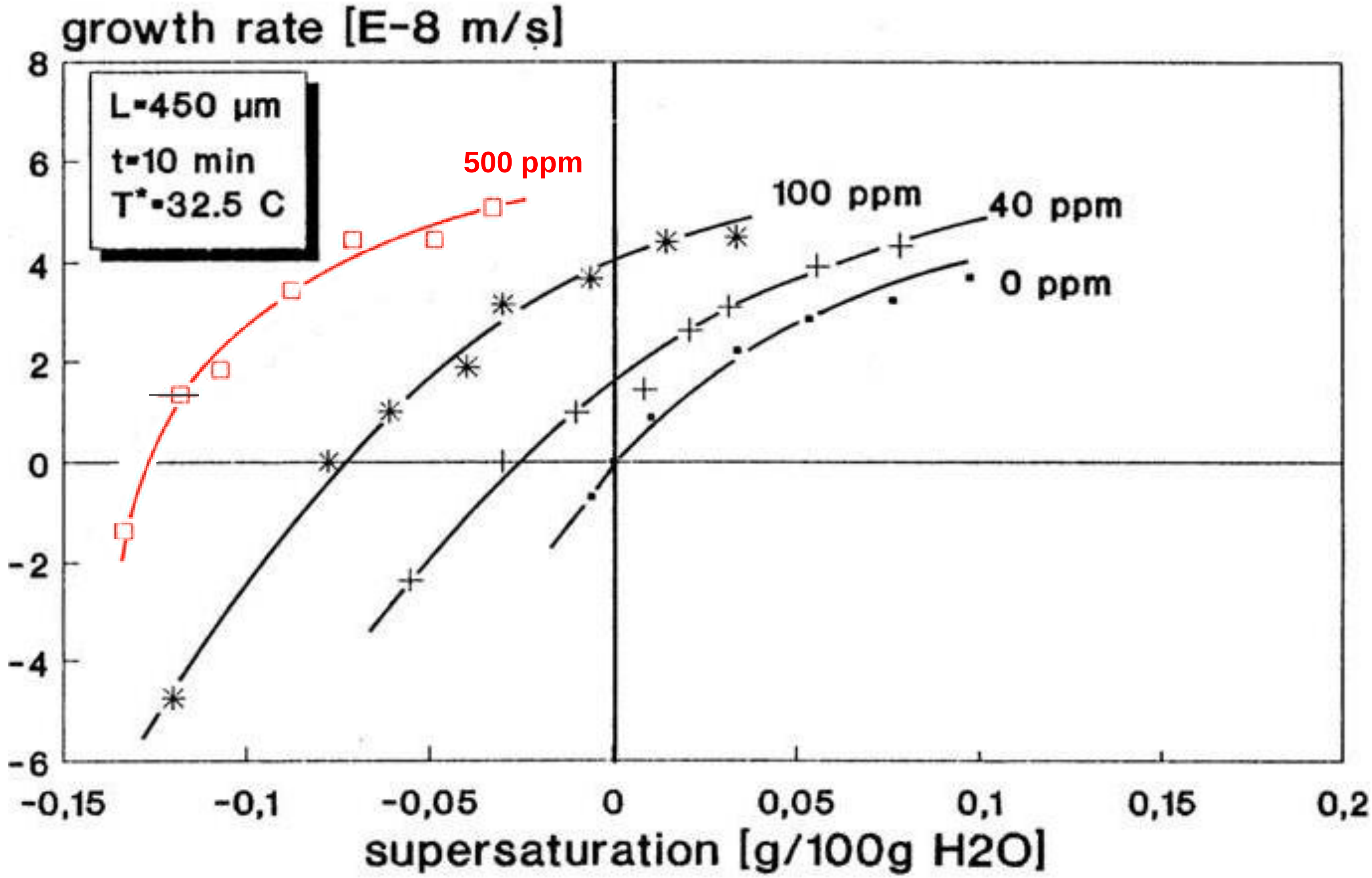
The effect of pH on the growth and dissolution rates of $MgSO_4 \cdot 7H_2O$ with saturation point correction (kinetic effect)

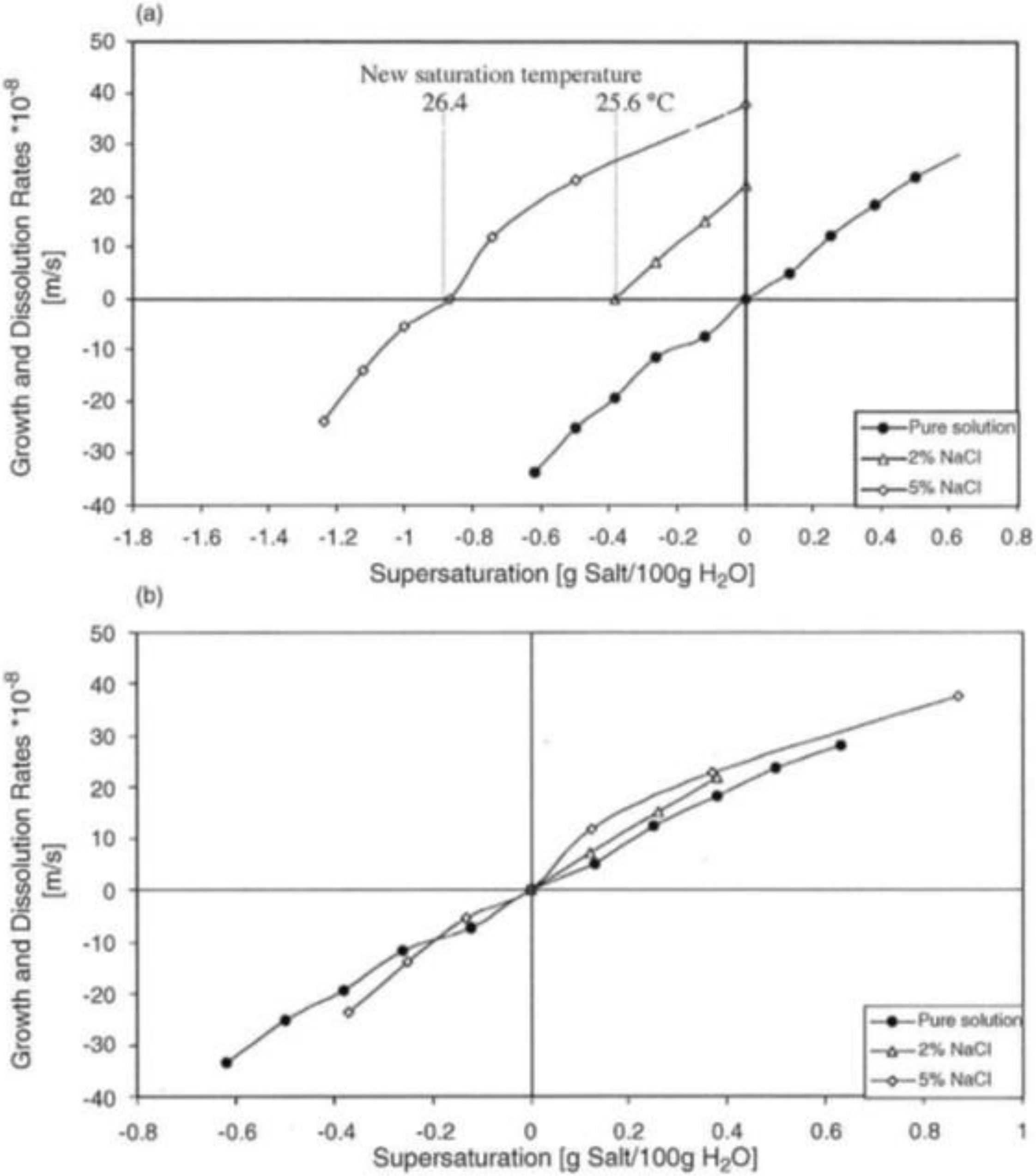


The effect of pH on the growth and dissolution rates of MgSO4 . 7H2O with saturation point correction (kinetic effect)



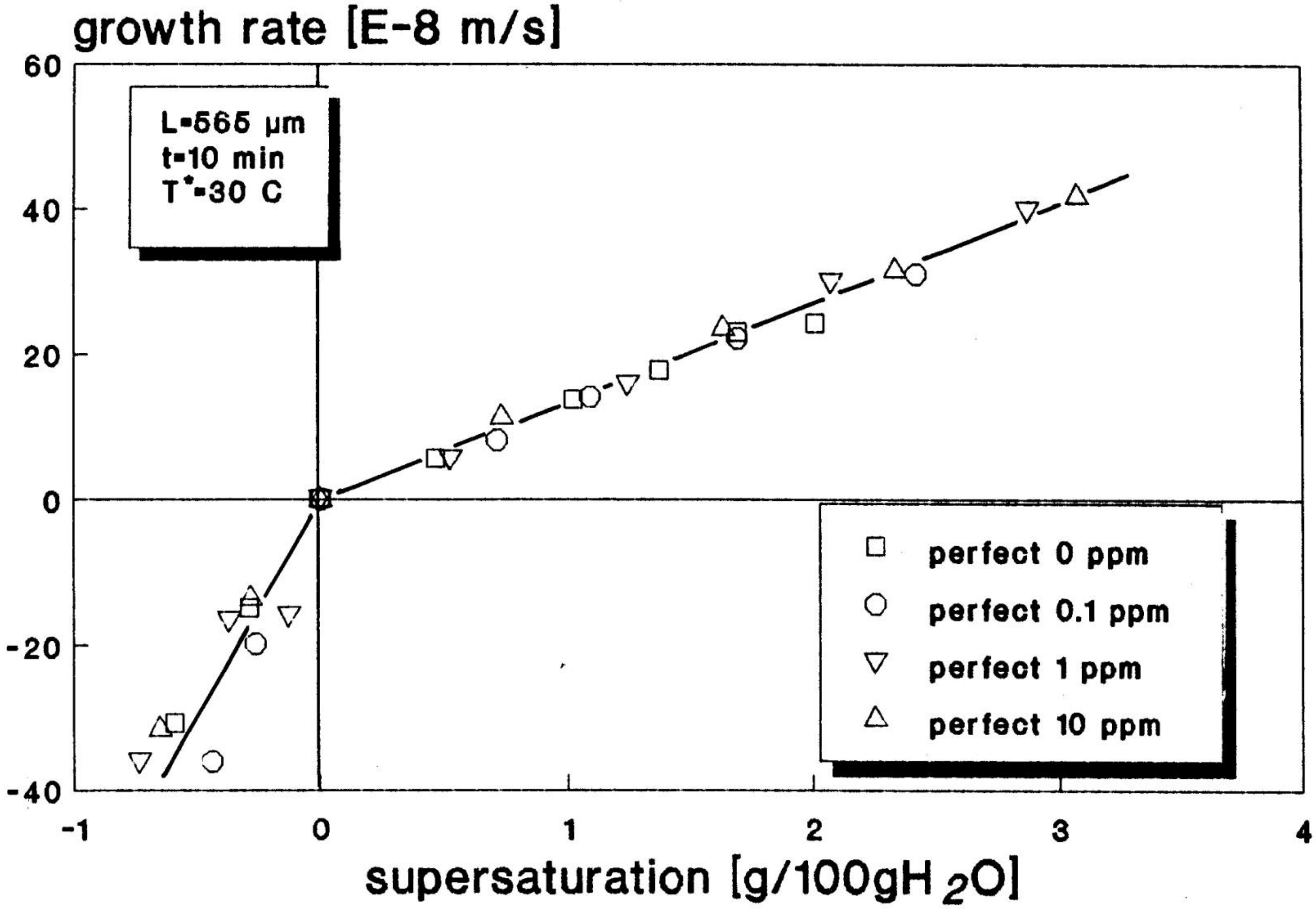
“Apparantly accelerated growth” of NaCl by the additive $MgCl_2 \cdot 6\ H_2O$





(a)
New saturation temperature
26.4
25.6 °C
Growth and Dissolution Rates *10^-8 [m/s]
Supersaturation [g Salt/100g H2O]
Pure solution
2% NaCl
5% NaCl
(b)
Growth and Dissolution Rates *10^-8 [m/s]
Supersaturation [g Salt/100g H2O]
Pure solution
2% NaCl
5% NaCl

Growth and dissolution rates of $KAl(SO_4)_2 \cdot 12H_2O$ in presence of $PbCl_2$



Effect of Supercooling on Nucleation and Growth Rate

Nucleation rate

$$J = k_n \Delta c_{max}^n$$

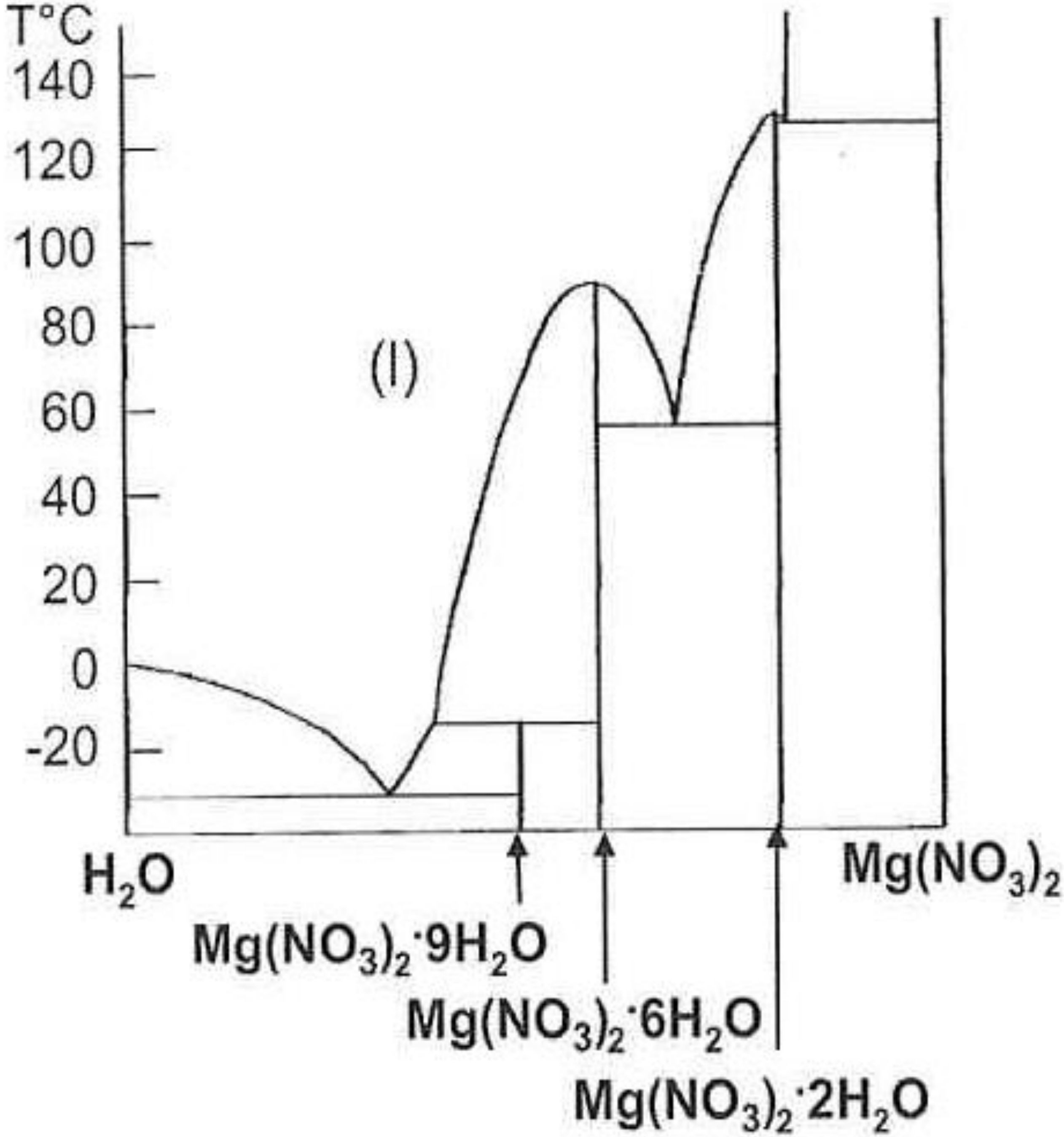
Growth rate

$$G = k_g \Delta c^g$$

with $\Delta c_{max} = \left(\frac{dc^*}{dt} \right) \Delta T_{max}$ and $\Delta c = c - c^*$

Thank you for your attention!

- **Polymorph**
- **Solvat (Hydrat)**



T°C
140
120
100
80
60
40
20
0
-20
(l)
H_2O
$Mg(NO_3)_2$
$Mg(NO_3)_2 \cdot 9H_2O$
$Mg(NO_3)_2 \cdot 6H_2O$
$Mg(NO_3)_2 \cdot 2H_2O$

Thermodynamically stable phases of organic and inorganic substances depend on:

- Temperature
- Pressure
- surrounding media:

  - air (relative humidity)
  - solvent (solubility)
- additional: mechanical stress (e.g. grinding)

→ **A change of these parameters often leads to metastable modifications**

Definition of polymorphism and solvation

- If a substance is capable of crystallizing into different, but ***chemically identical*** crystalline forms, it is said to exhibit: ***polymorphism***.
- If the anhydrous/non-solvate part is identical, but the crystalline forms ***differ in the amount of water/solvent*** in the lattice, the crystalline form is said to exhibit: ***solvation***.

„No rules exist that allow the prediction of whether a compound will exhibit polymorphism...“

Stephen R. Byrn: „Solid-State Chemistry of Drugs“,
Academic Press, New York, 1982

Industrial relevance of polymorphs and solvates

- The change in physical properties such as:

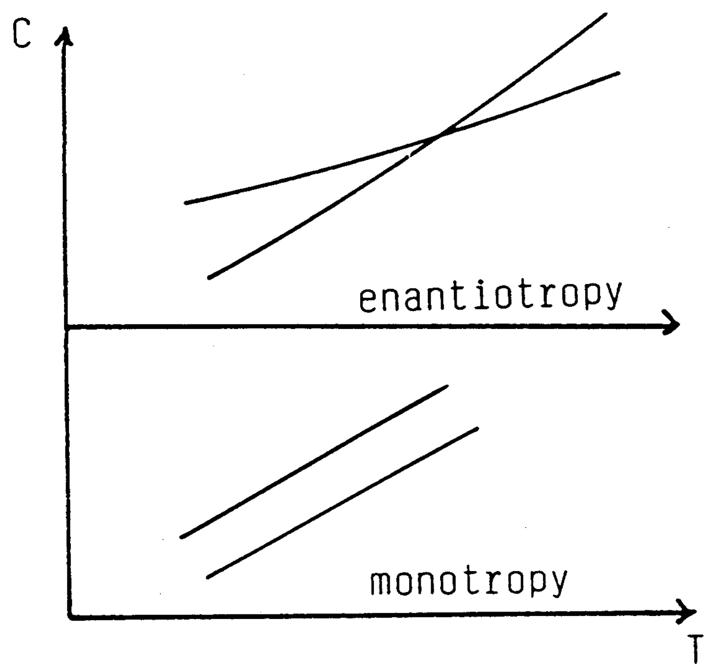
  - bioavailability
  - dissolution behaviour
  - suspension syringeability
  - chemical and physical stability
  - solid-liquid-separation
  - drying
  - packaging
  - tabletting

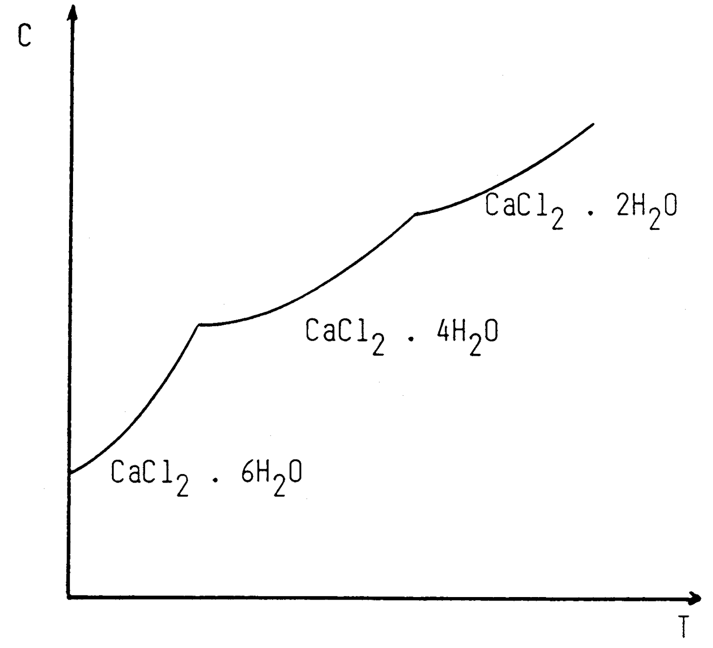
Important:

→ Control of the experimental parameters (temperature, pressure, concentration) ***to avoid or generate metastable modifications***

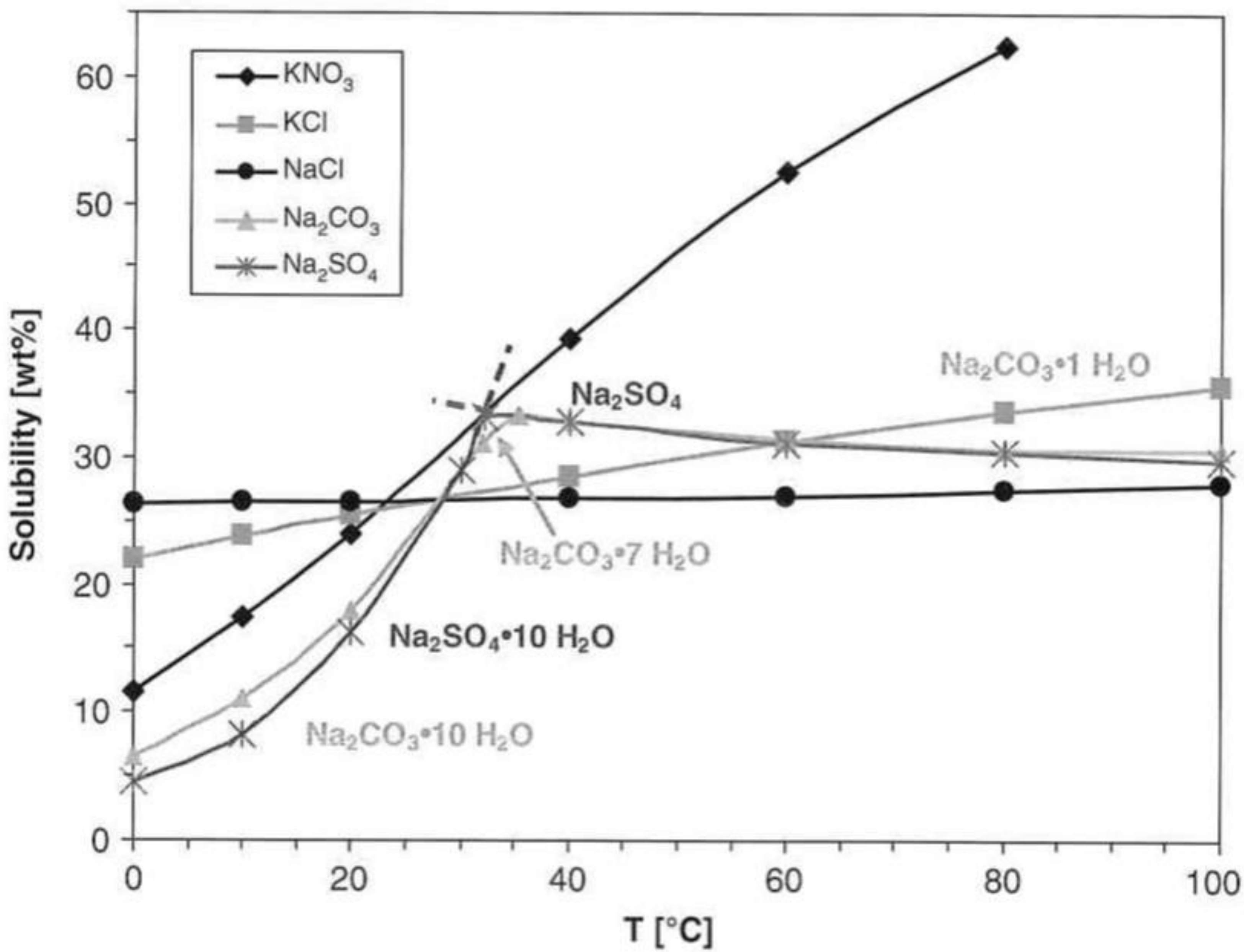
Phase transformations: principles

(left) schematic solubility diagrams for polymorphic systems exhibiting enantiotropic and monotropic phase transformations, (right) schematic solubility diagram for $CaCl_2$- hydrate structures exhibiting enantiotropic phase transformations

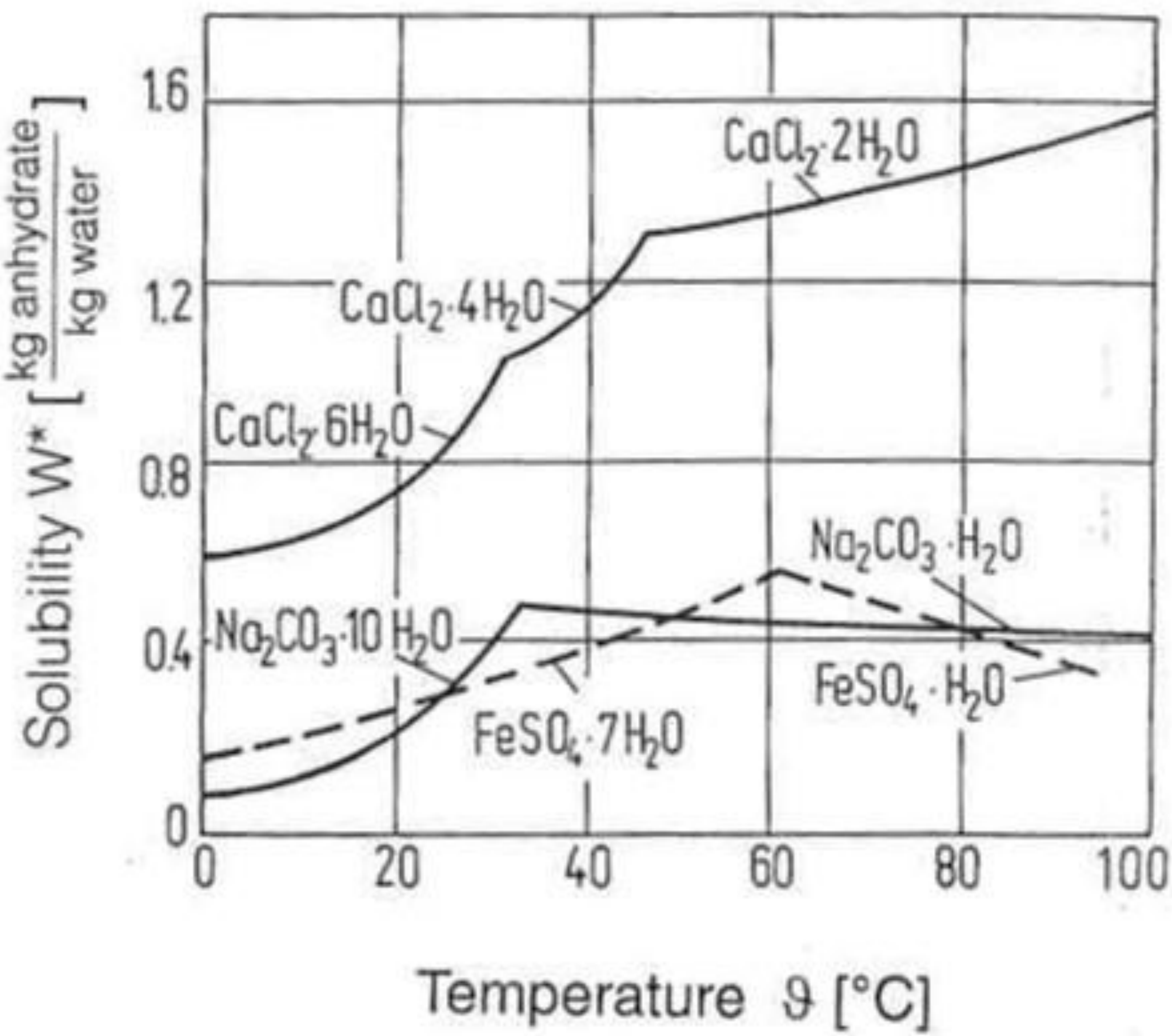




Mohnicke, M.: *On the control of riboflavin (vitamin B2) crystal structures*, Dissertation, Martin-Luther-Universität Halle-Wittenberg (2006), Aachen, Shaker Verlag 2007, ISBN: 978-3-8322-6064-4.



KNO3
KCl
NaCl
Na2CO3
Na2SO4
Na2CO3•1 H2O
Na2SO4
Na2CO3•7 H2O
Na2SO4•10 H2O
Na2CO3•10 H2O
Solubility [wt%]
T [°C]
0
10
20
30
40
50
60
0
20
40
60
80
100

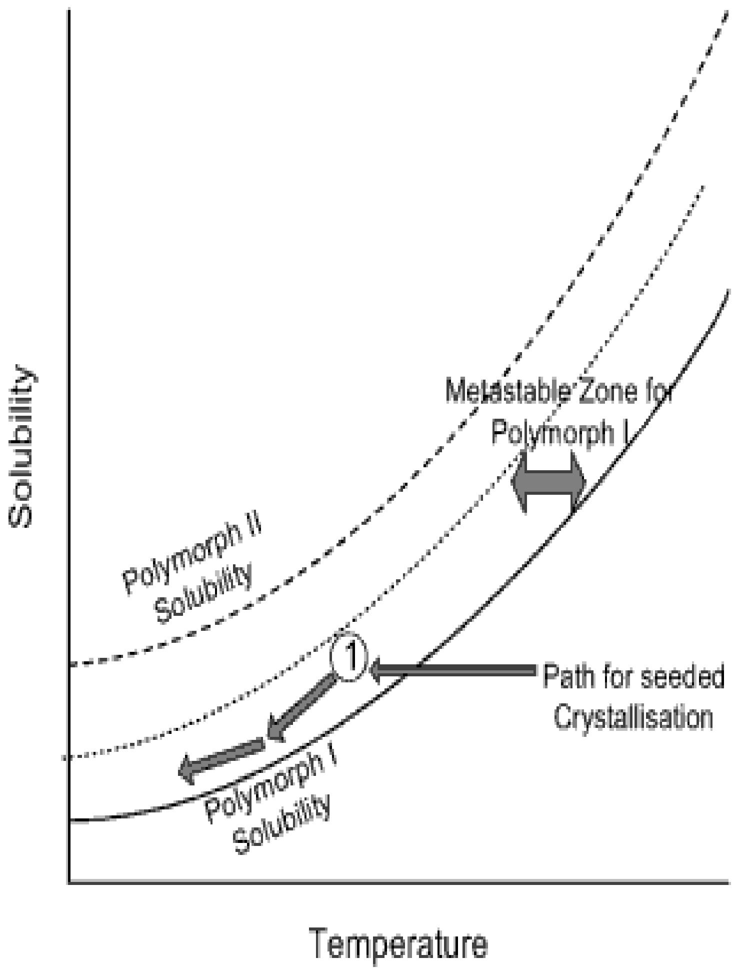

Solubility W* [kg anhydrate / kg water]
1.6
1.2
0.8
0.4
0
0
20
40
60
80
100
Temperature ϑ [°C]
CaCl₂·2H₂O
CaCl₂·4H₂O
CaCl₂·6H₂O
Na₂CO₃·H₂O
Na₂CO₃·10 H₂O
FeSO₄·H₂O
FeSO₄·7H₂O

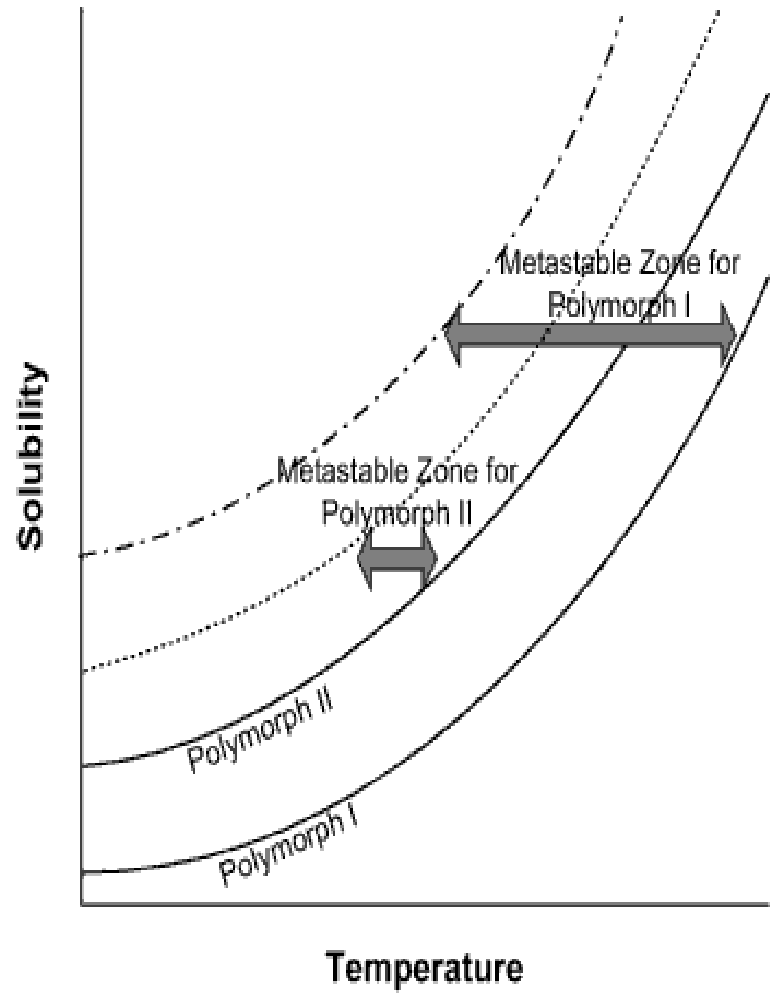
Different units t o express a solution concentration of 10 g Na_2SO_4 in 100 g water

(Substance available as anhydrous Na_2SO_4 and Glauber's salt ($Na_2SO_4 \cdot 10\ H_2O$))

- 9.1 g Na_2SO_4 / 100 g solution = 9.1 wt%
- 12.7 mol Na_2SO_4 / 1000 mol H_2O
- 0.7 mol Na_2SO_4 / 1000 g H_2O
- 26 g Na_2SO_4- 10 H_2O / 100 g H_2O
- 20.6 g $Na_2SO_4 \cdot 10\ H_2O$ / 100 g solution

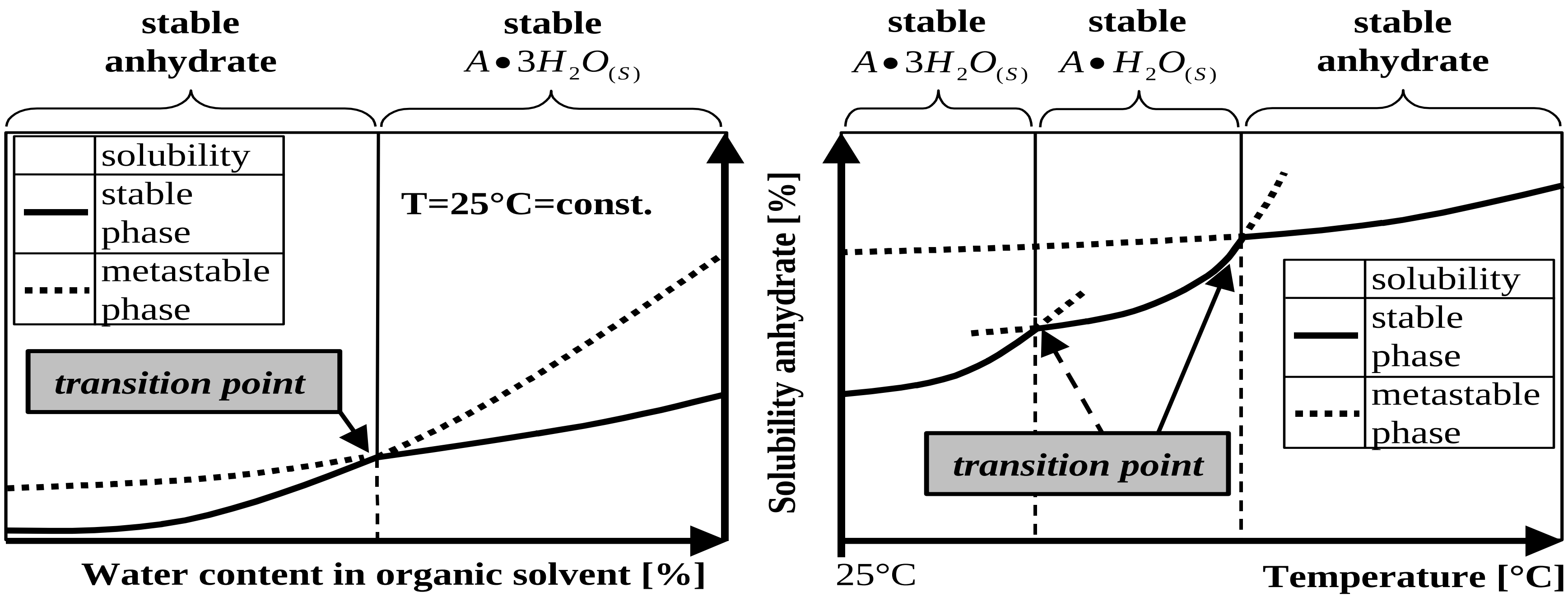
Different possibilities of MSZW of monotropic system, overlapped MSZ of form I (right)





Cussler, L.:Chemical Product Design, Cambridge, 2001.

Comparison of *transition points* in binary and ternary systems

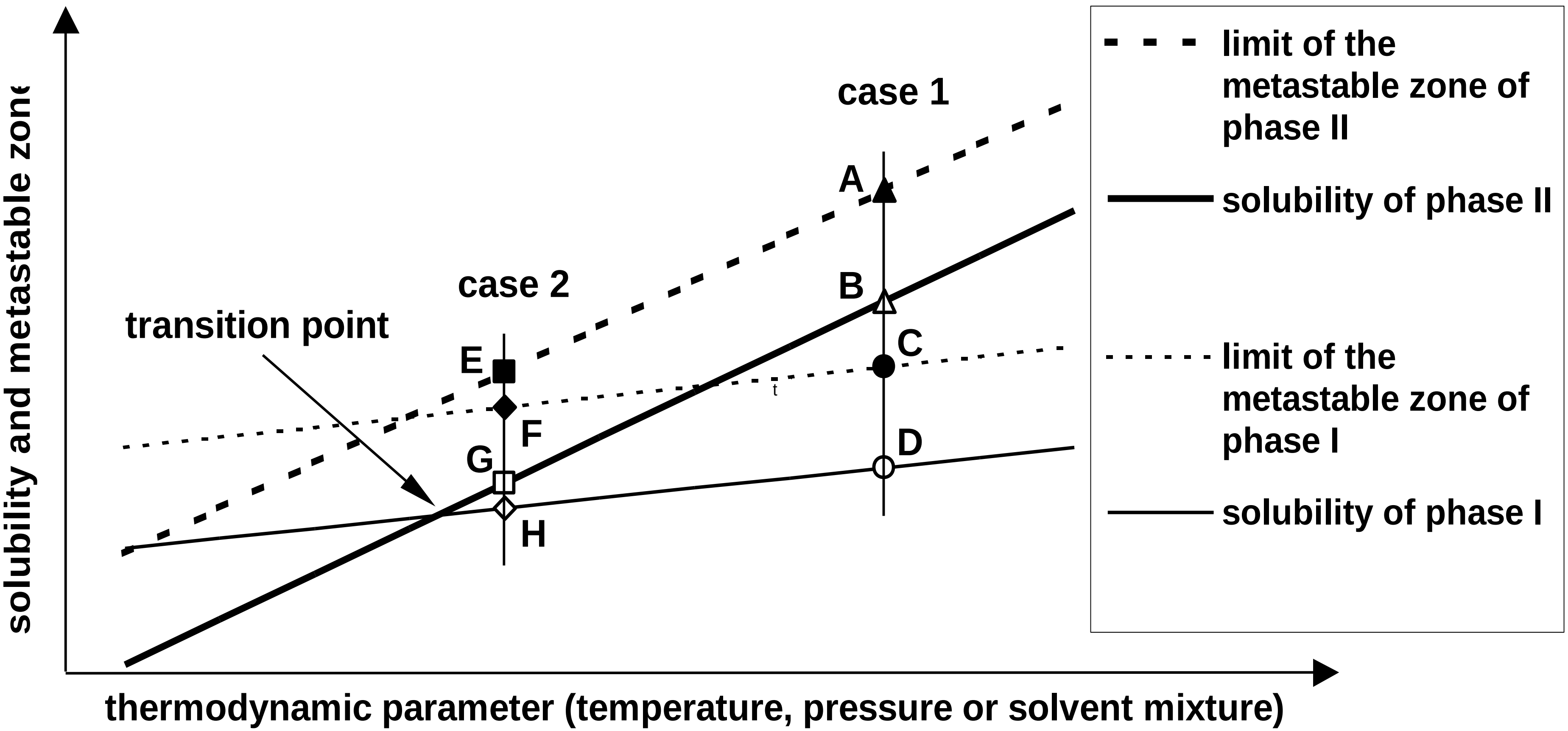


a) ternary system
(anhydrate *A* - water - organic solvent),
p, T =const.

b) binary system
(anhydrate *A* - water),
p = const.

Nordhoff, St.: *Zur Pseudopolymorphie unter Lösungsmitteleinfluss*, Dissertation, Universität Bremen, Aachen, Shaker Verlag 1999, ISBN: 978-3-8265-6303-4

Metastable Zone

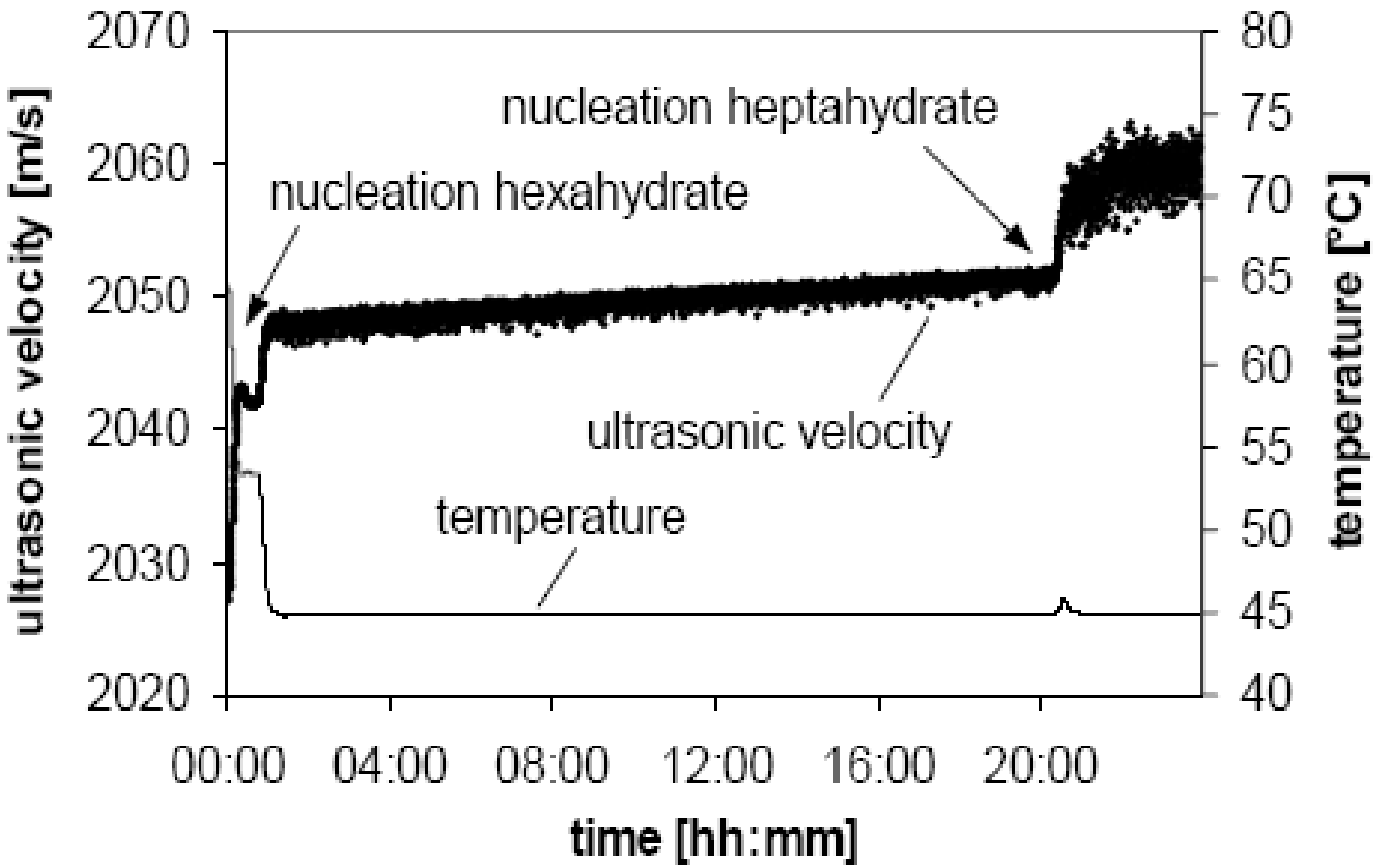


Nordhoff, St.: *Zur Pseudopolymorphie unter Lösungsmitteleinfluss*, Dissertation, Universität Bremen, Aachen, Shaker Verlag 1999, ISBN: 978-3-8265-6303-4.

Example

Solvates with additives

- Phase transformation of magnesium sulfate heptahydrate to the hexahydrate

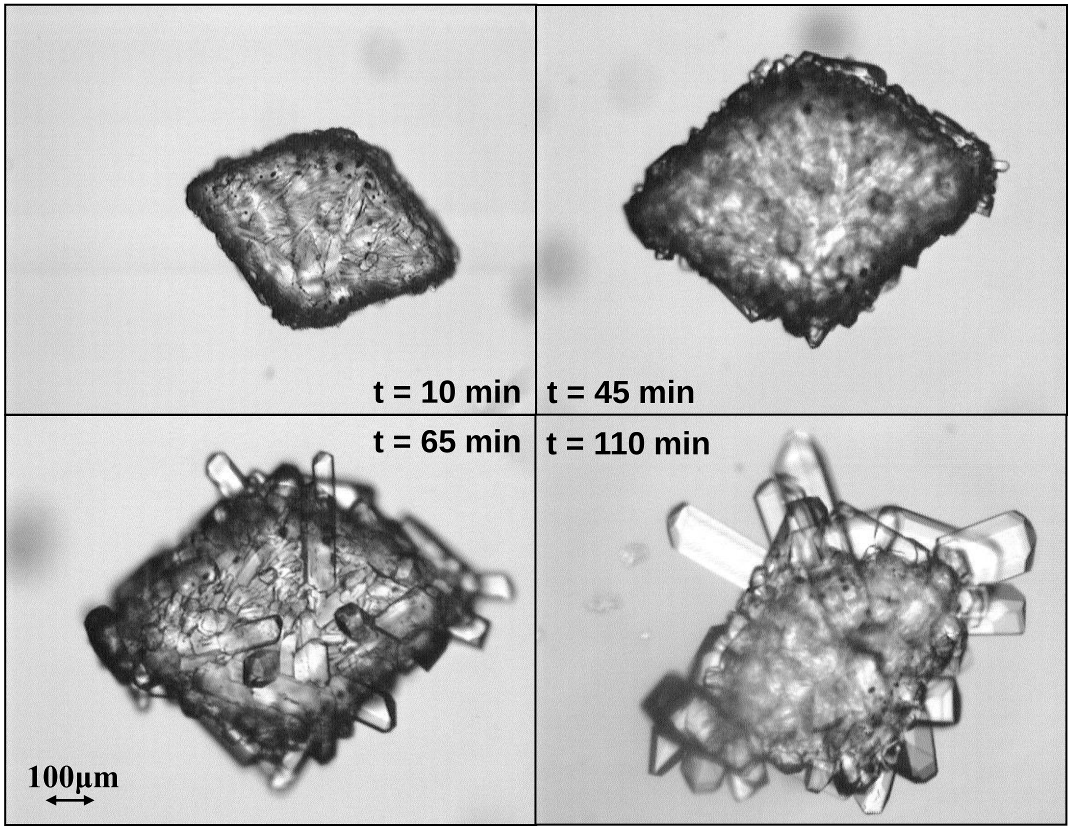


Strege, C.: *On (Pseudo-) Polymorphic Phase Transformations*, Dissertation, Martin-Luther-Universität Halle-Wittenberg, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/04/04H318/index.htm, 2004.

Example

making use of the kinetic - time related processes - not only thermodynamics

- Phase transformation of magnesium sulfate hexahydrate to the heptahydrate



Strege, C.: *On (Pseudo-) Polymorphic Phase Transformations*, Dissertation, Martin-Luther-Universität Halle-Wittenberg, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/04/04H318/index.htm, 2004.

Example

making use of the kinetic - time related processes - not only thermodynamics

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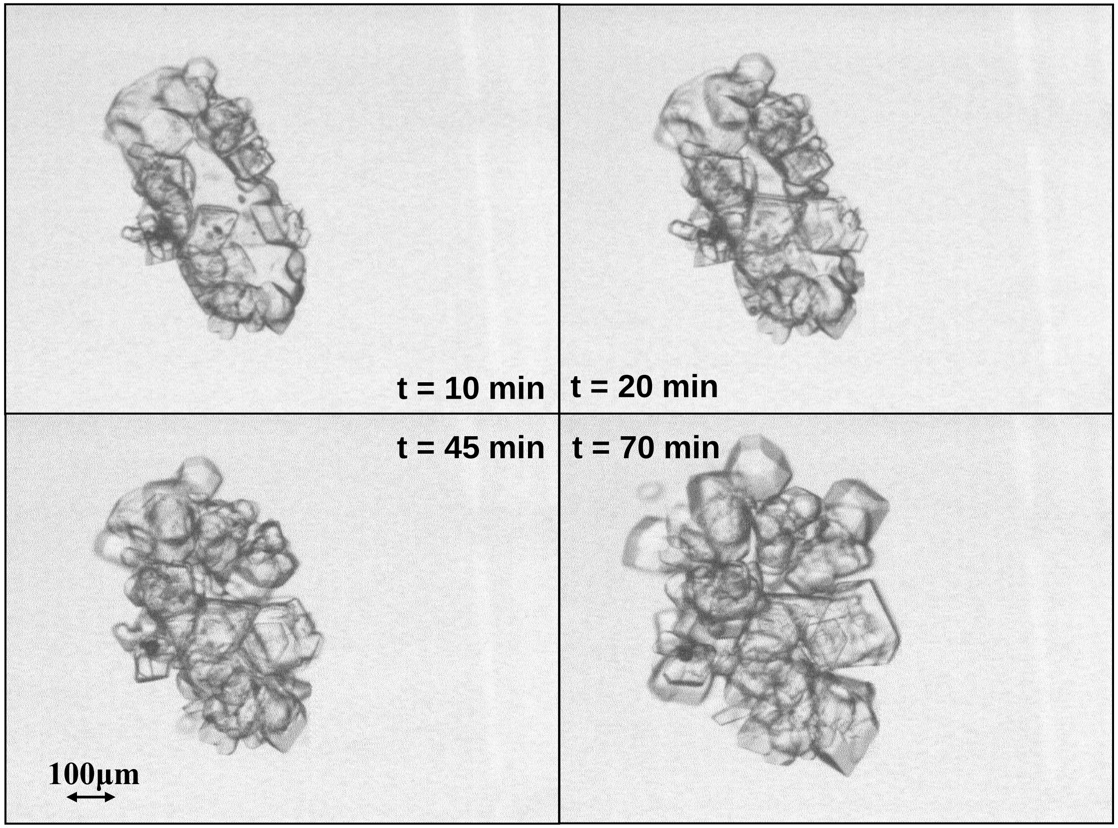
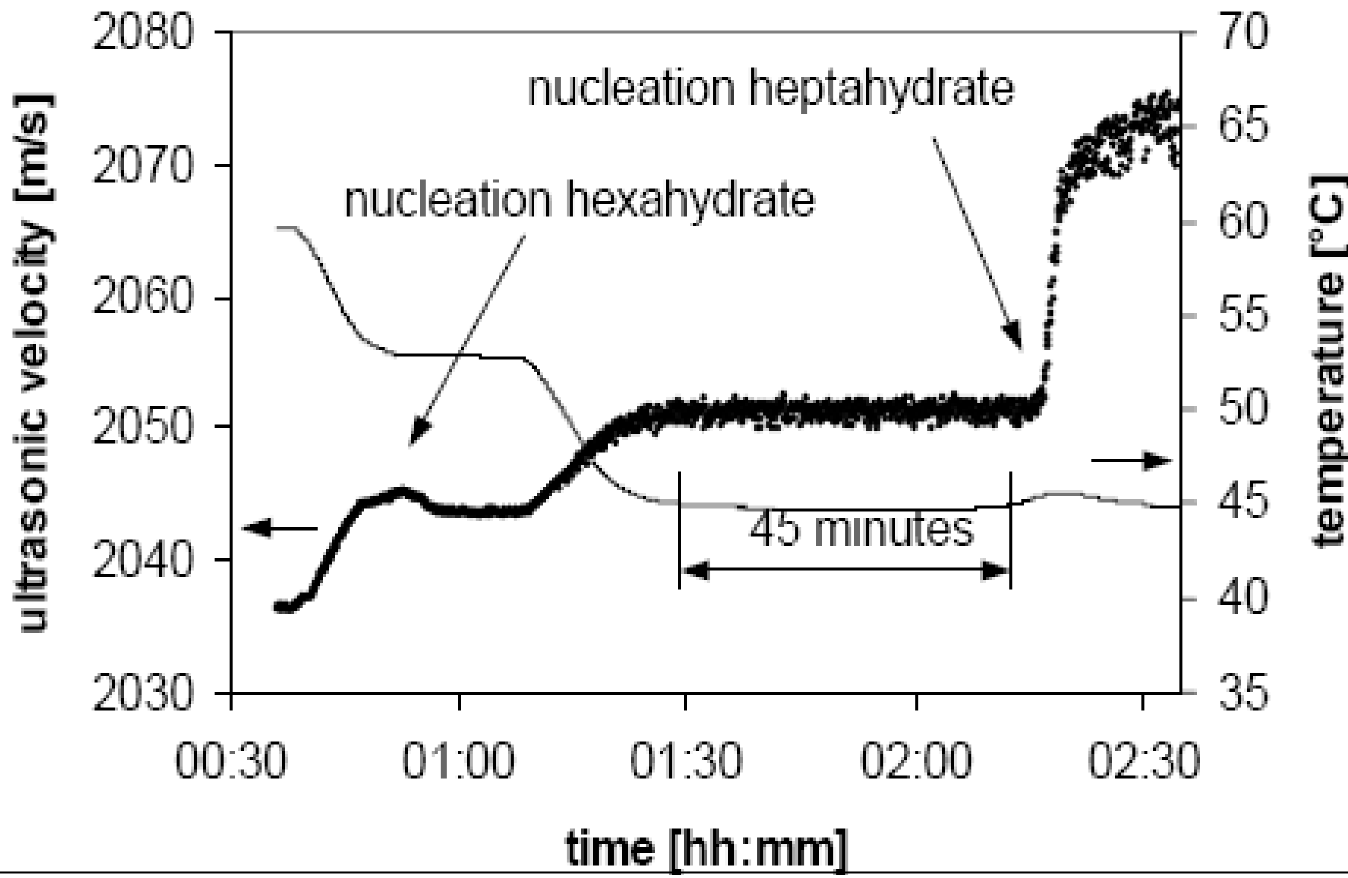


Strege, C.: *On (Pseudo-) Polymorphic Phase Transformations*, Dissertation, Martin-Luther-Universität Halle-Wittenberg, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/04/04H318/index.htm, 2004.

Example

Solvates with additives

- Phase transformation of magnesium sulfate hydrate with 0.5 wt% KCl

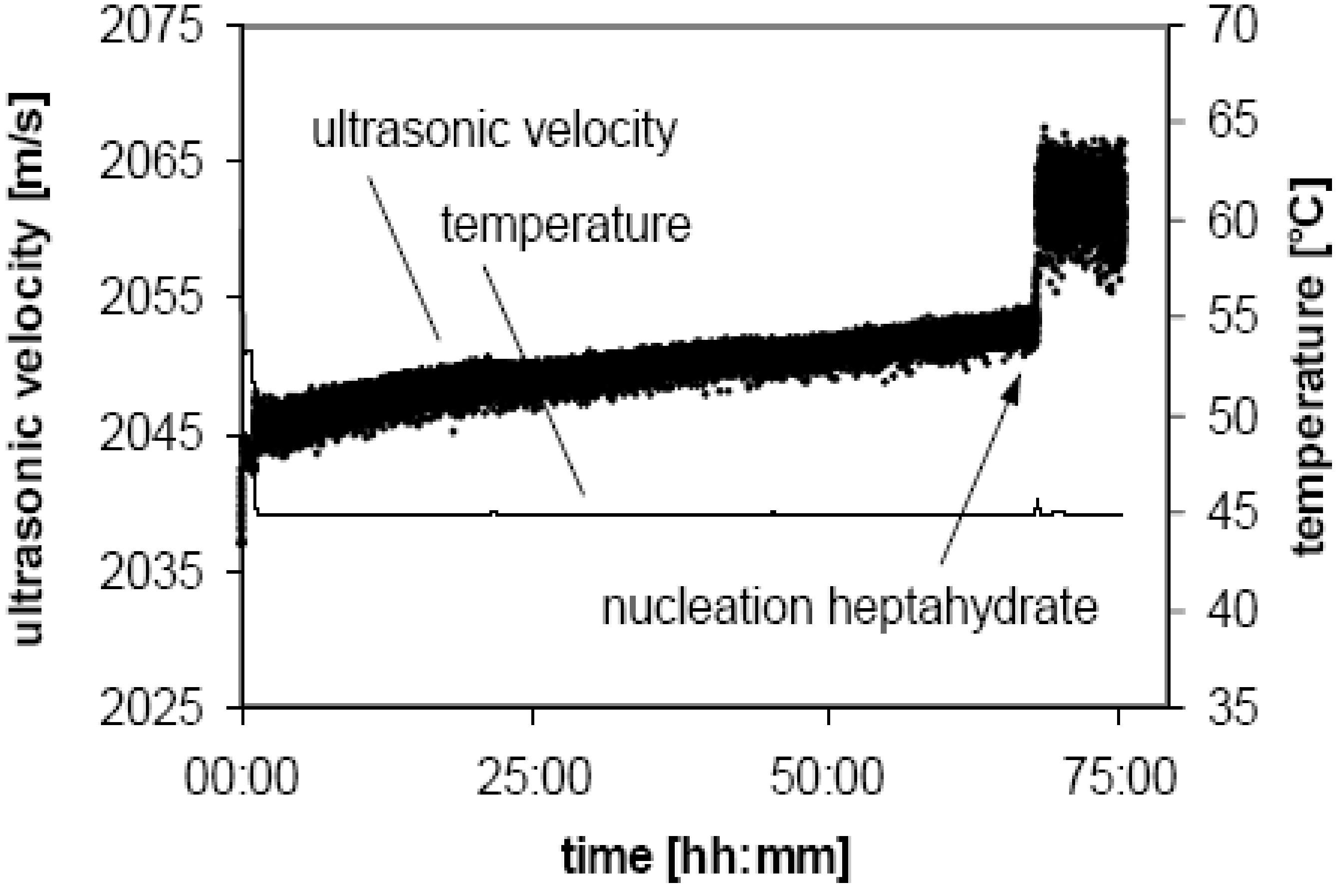


Strege, C.: *On (Pseudo-) Polymorphic Phase Transformations*, Dissertation, Martin-Luther-Universität Halle-Wittenberg, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/04/04H318/index.htm, 2004.

Example

Solvates with additives

- Phase transformation of magnesium sulfate hydrate with 0.5 wt% borax

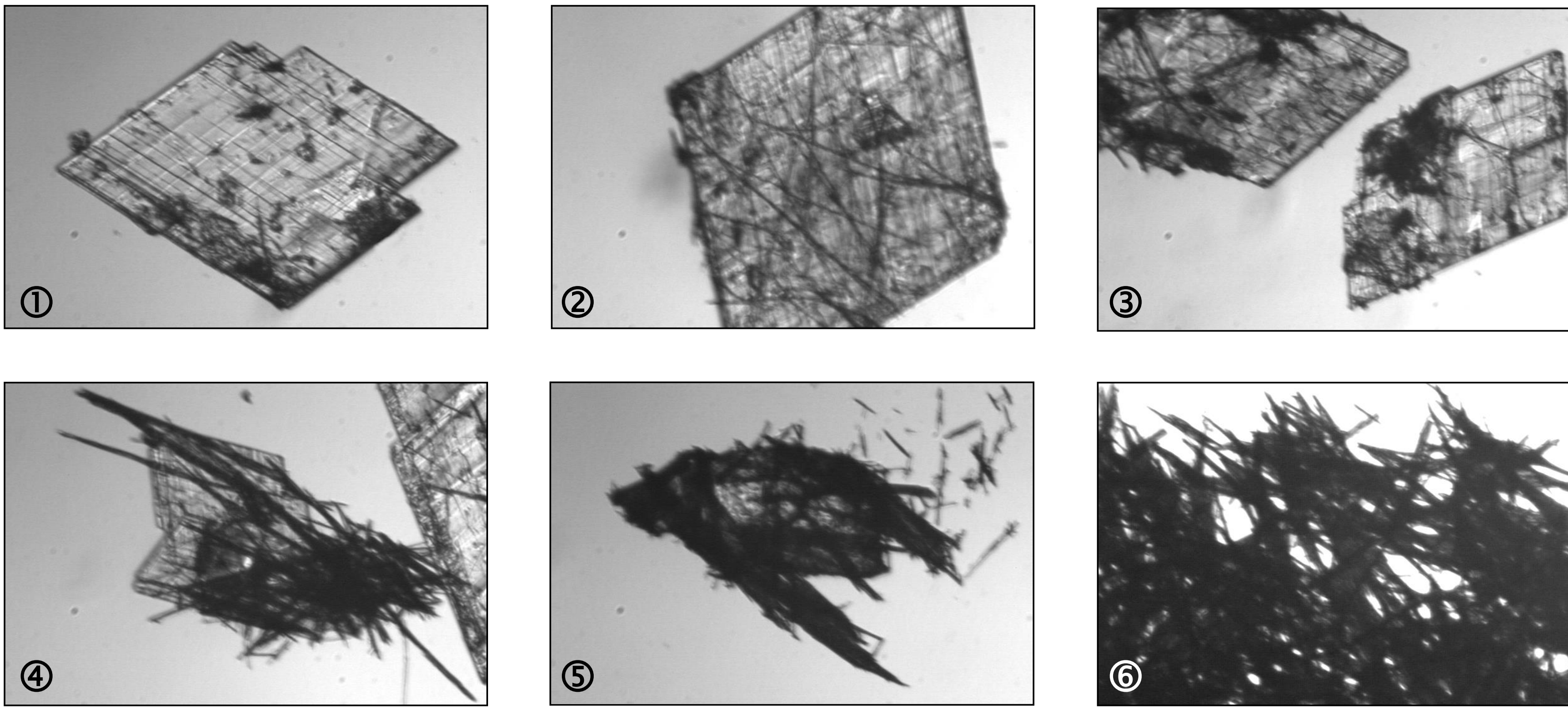


Strege, C.: *On (Pseudo-) Polymorphic Phase Transformations*, Dissertation, Martin-Luther-Universität Halle-Wittenberg, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/04/04H318/index.htm, 2004.

Example

making use of the kinetic - time related processes - not only thermodynamics

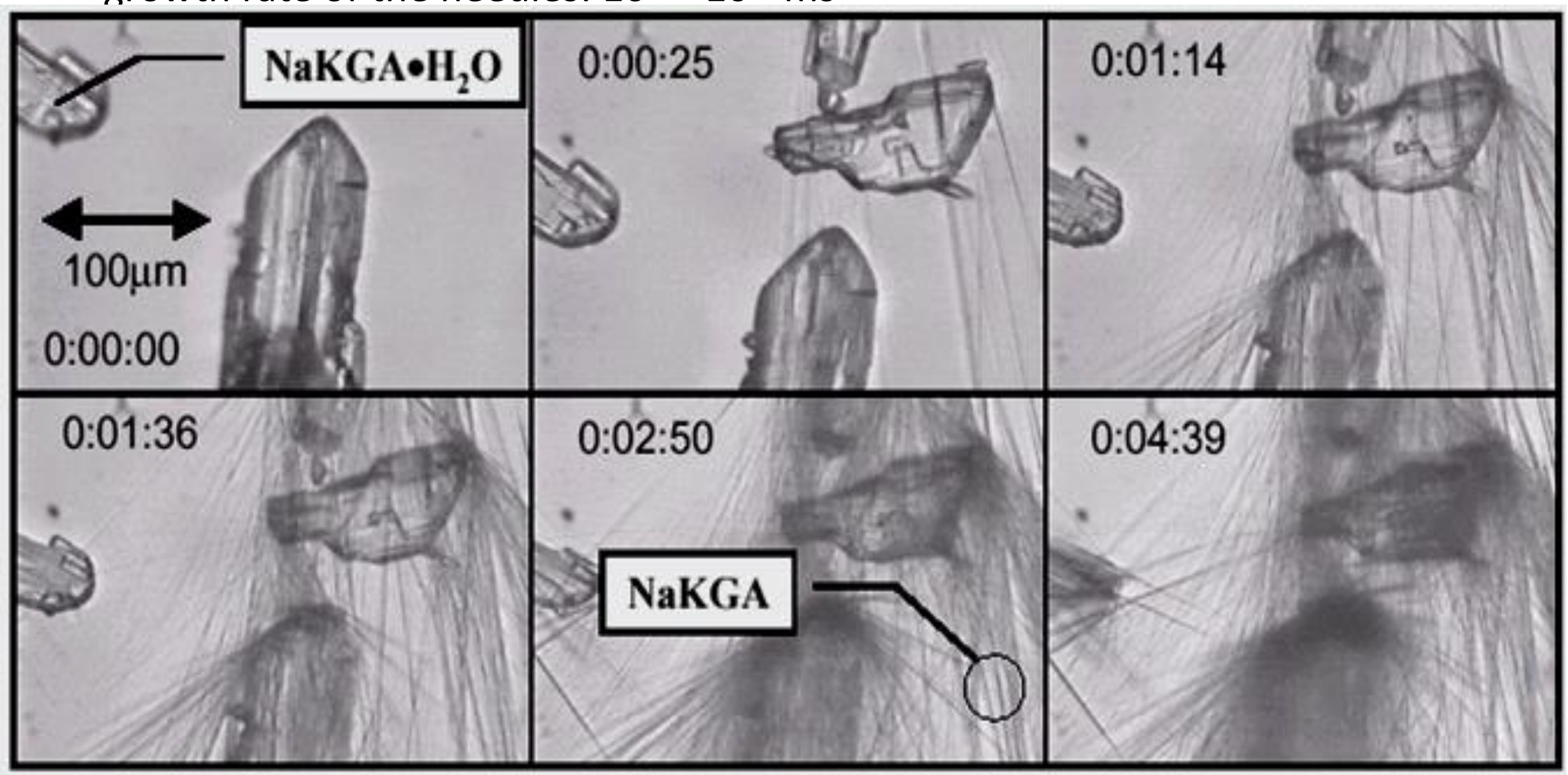
- Phase transformation of anhydrous l-phenylalanine to the monohydrate [1]



[1] Strege, C.: *On (Pseudo-) Polymorphic Phase Transformations*, Dissertation, Martin-Luther-Universität Halle-Wittenberg, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/04/04H318/index.htm, 2004.

Phase Transition

- Sodium-2-keto-L-gulonate-monohydrate (skgm)
- growth rate of the needles: 10^{-5} - 10^{-4} ms^{-1}

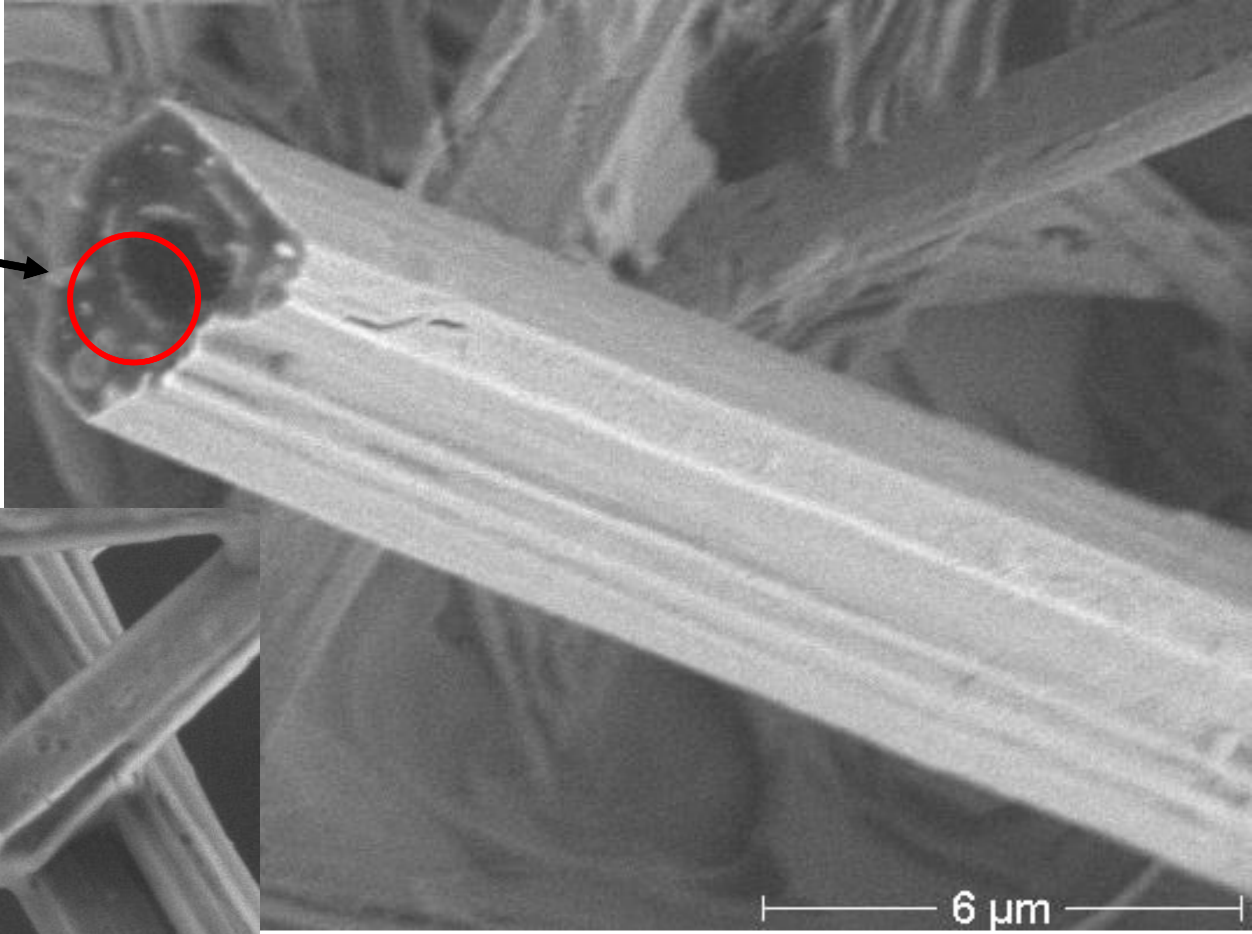


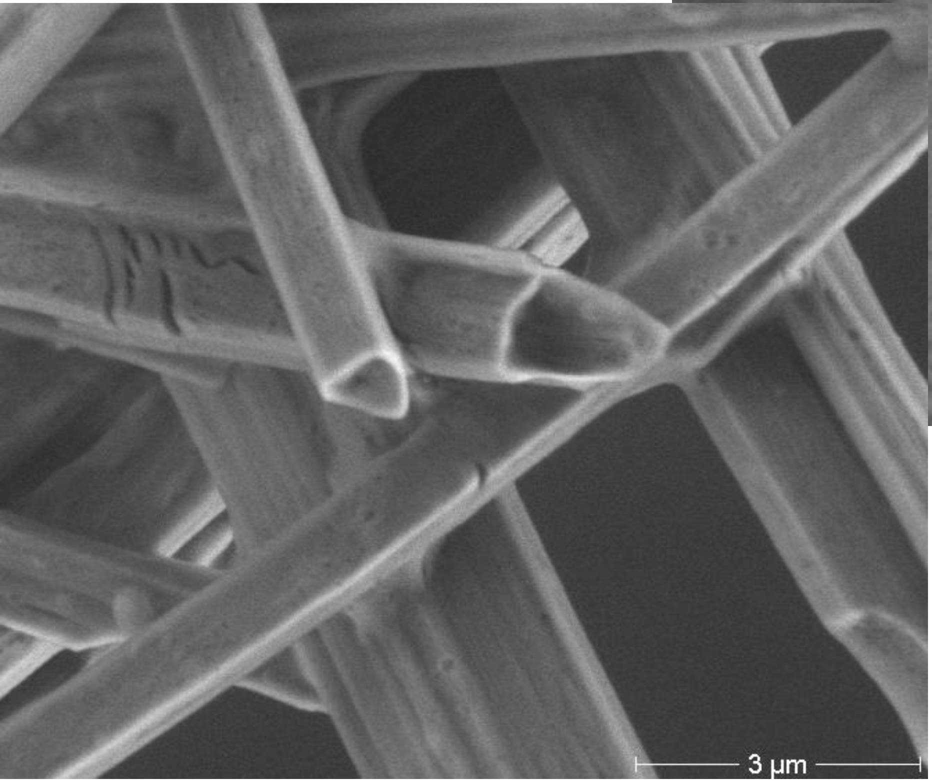
Needle growth during the transformation of skgm in methanol

Bechtloff, B.: *Gezielte Beeinflussung der Kinetik von Fest-Flüssig-Reaktionskristallisationen*, Dissertation, Martin-Luther-Universität Halle-Wittenberg, 2002, Onlineveröffentlichung: http://sundoc.bibliothek.uni-halle.de/diss-online/02/02H105/index.htm.

Solvent mediated phase transitions

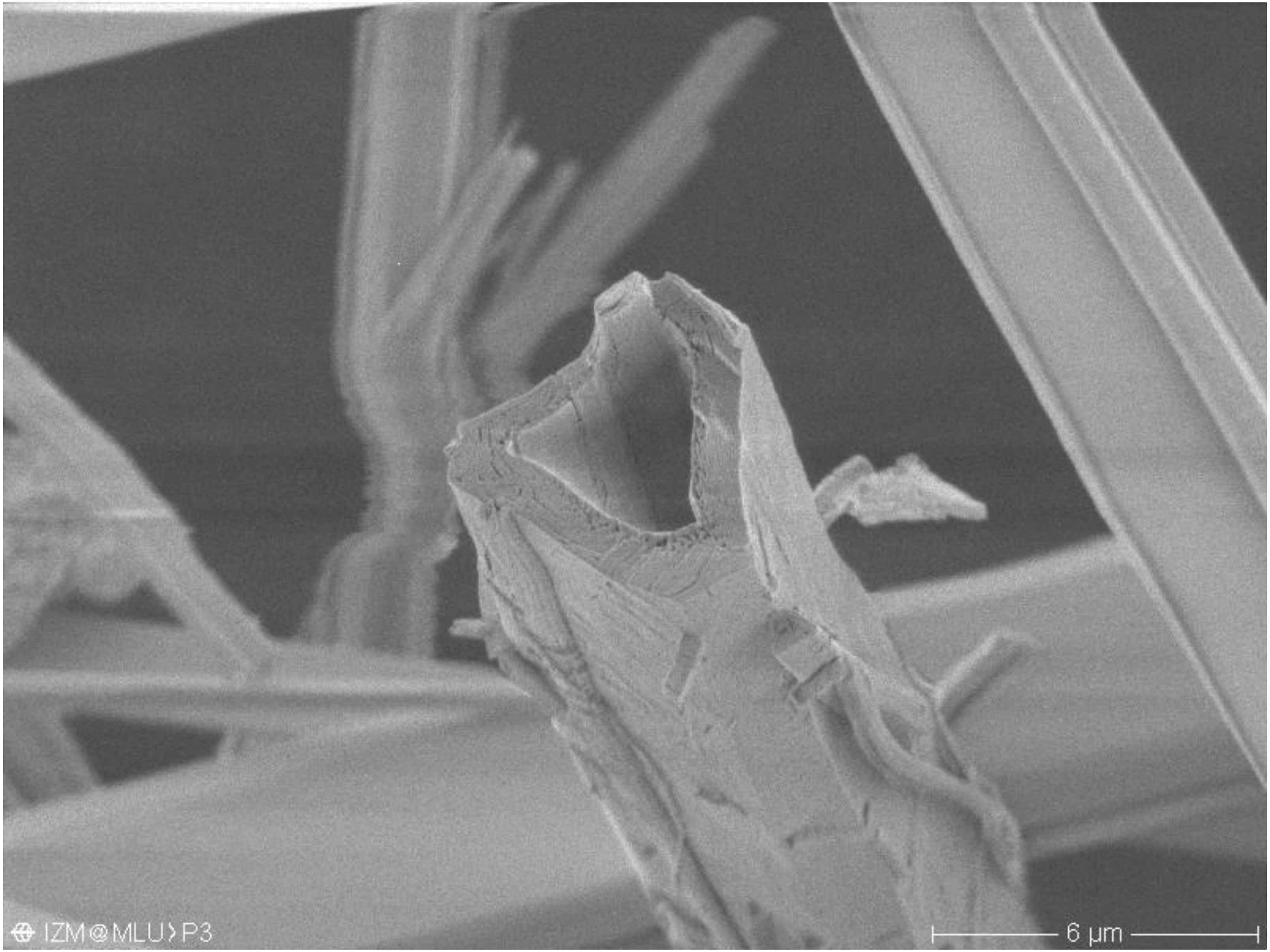
100g methanol (≈150ml, left); 10g NaKGA monohydrate (centre), after suspending the hydrate in methanol (right)

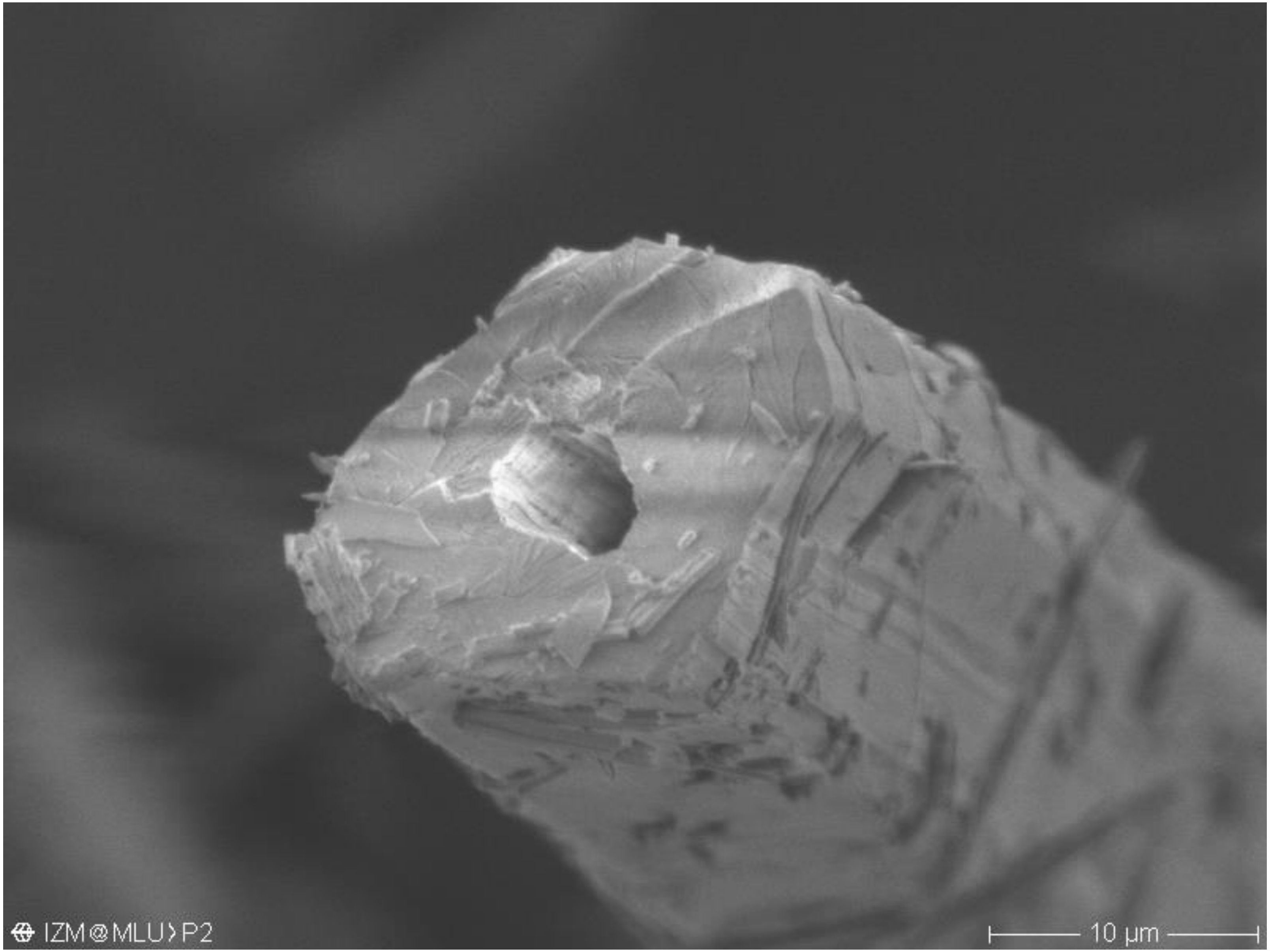




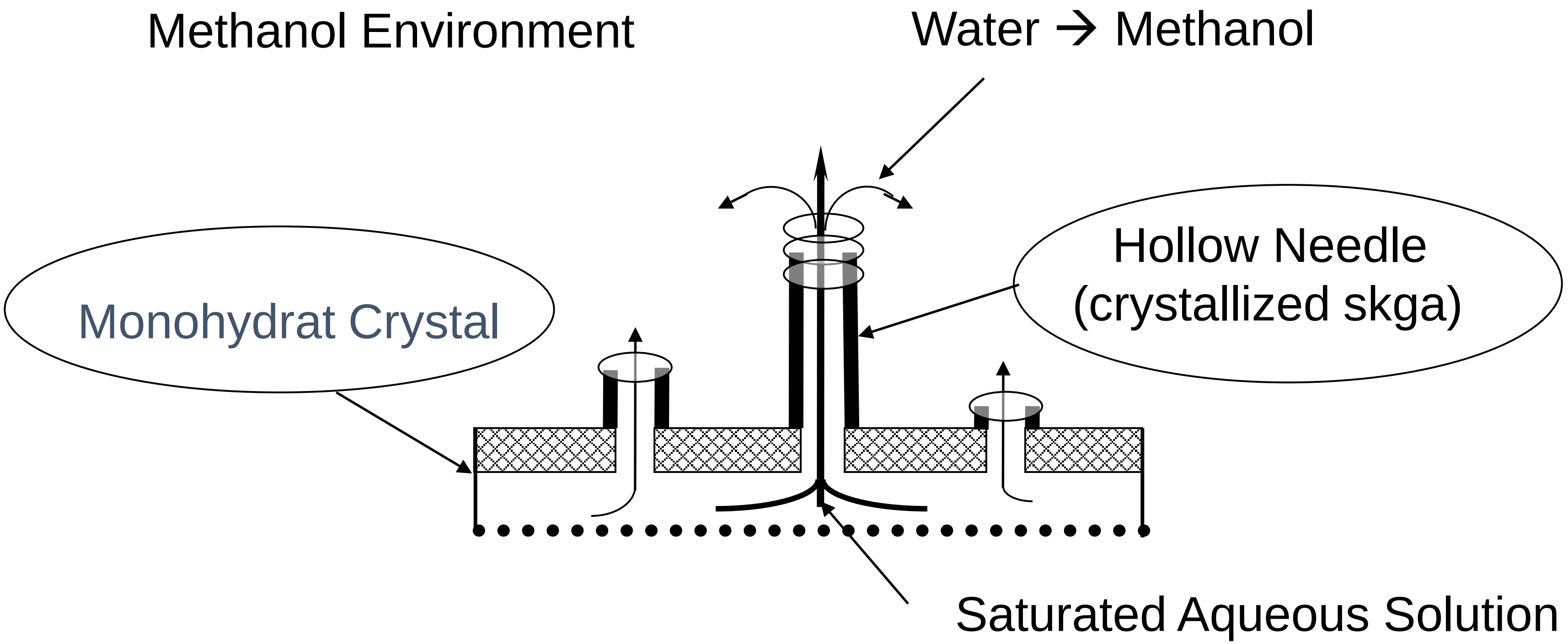
SEM images of hollow skga needles.

Dette, S.S.: *Kristalline Röhren - Erzeugt durch die Dehydratation in organischen Lösungsmitteln*, Dissertation, Martin-Luther-Universität Halle-Wittenberg, Aachen, Shaker Verlag 2009, ISBN: 978-3-8322-8051-2.


IZM @ MLU › P3
6 µm

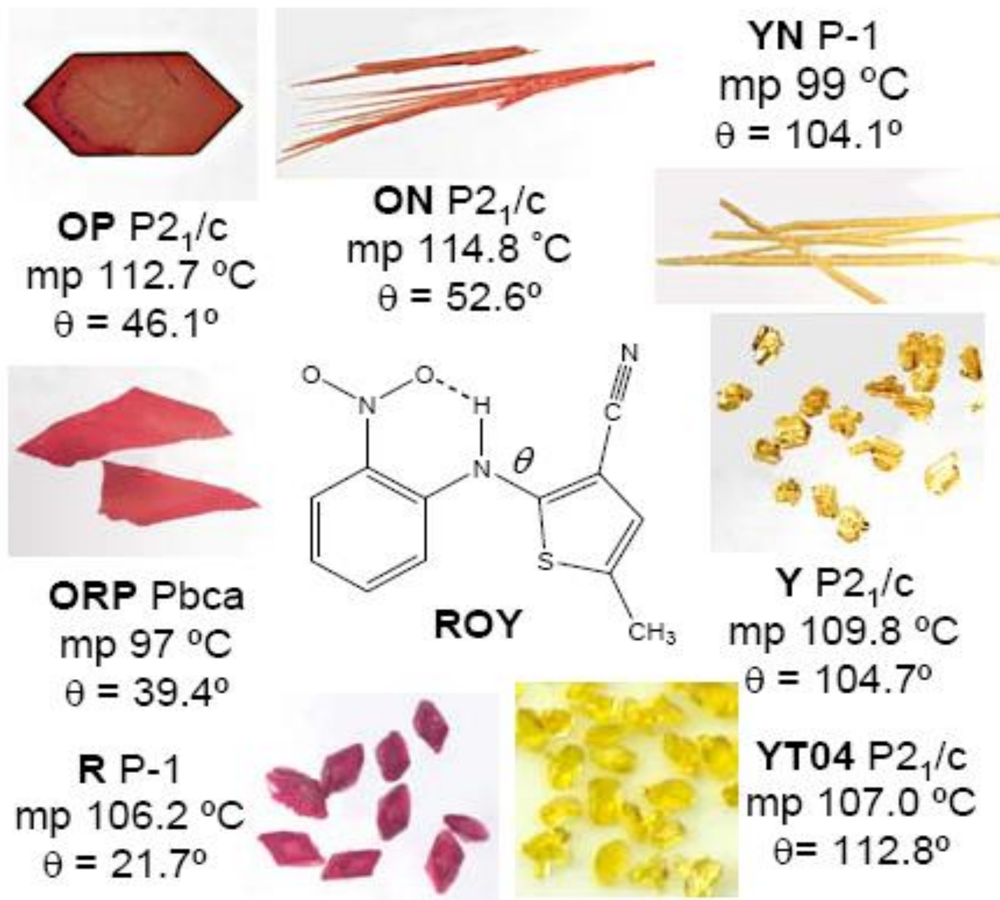

IZM@MLU›P2
10 µm

Dehydration in Methanol



Color difference between polymorphs of ROY

Olanzapine



Guo, J.: *Crystallization of Polymorphs: A Case Study on Astaxanthin and Apocarotenoic Ester,* Dissertation, Martin-Luther-Universität Halle-Wittenberg, 2009.

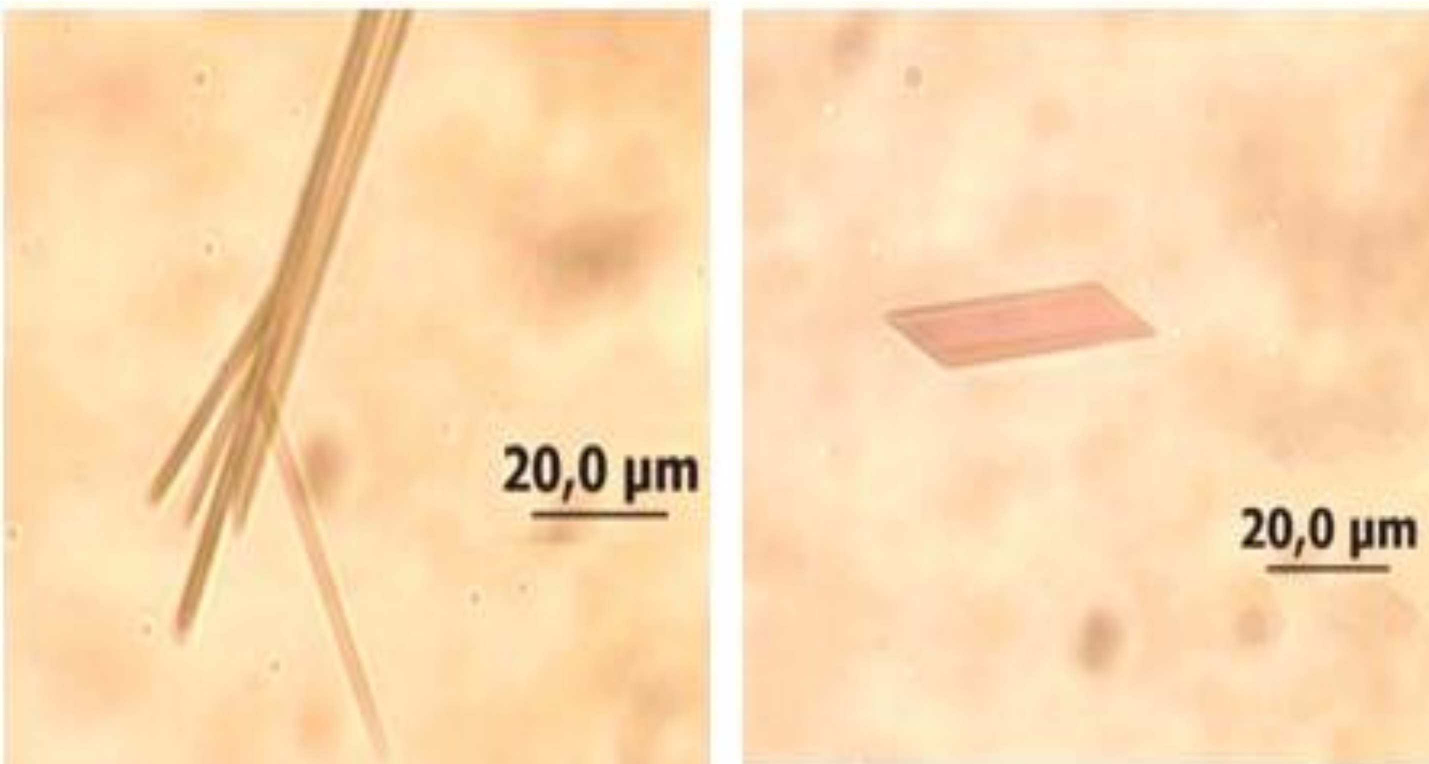
Yu, L., Chen, S., Xi, H.: *Cross Nucleation between ROY Polymorphs*, J. Am. Chem. Soc., 127 (2005), 17439 – 17444.

Techniques available for the examination of polymorphs

Method	Data measured	Feature obtained	Literature
Optical microscopy	Refractive index Inherent and polarization colour Interfacial angles	Particle shape and size, Surface characteristics Inclusions Symmetry elements	HAR 1960, 1964, McC 1978 McL 1990, STR 1997, BER 1998
Solubility analysis	Amount dissolved per volume and time (in different solvents)	Dissolution kinetics Saturation solubility Phase transition point	BUR 1982, BRI 1999, GU 2001
Moisture sorption/ desorption isotherms	Change of mass versus variable RH%	Hygroscopy Study of solvation and desolvation Prediction of handling properties	???
DSC	Heat flow versus temperature	Study of glass transition, melting and recrystallization point	GIR 1995, 1999, 2001, BUR 1990
TG	Change of mass versus temperature	Study of solvates Release and stability testing	CHE 2000, BRI 1995
FT-IR, ATR, DRIFT	IR-spectrum	Chemical information Phase characterization	KUH 1976, BRI 1997, GIR 1990, 1999,
Raman	Raman-spectrum	Chemical information Phase characterization	TUD 1993, TER 1994, GU 1995
Solid state NMR	Magnetic resonance	Chemical information Phase characterization	BUG 1993, HAR 1993, BRI 1997, BYR 1999
X-ray diffraction	Diffractogram	Chemical information Phase characterization structure determination Crystallinity measurement	AZA 1958, BIS 1989, ZEV 1995, JEN 1996, STE 2001

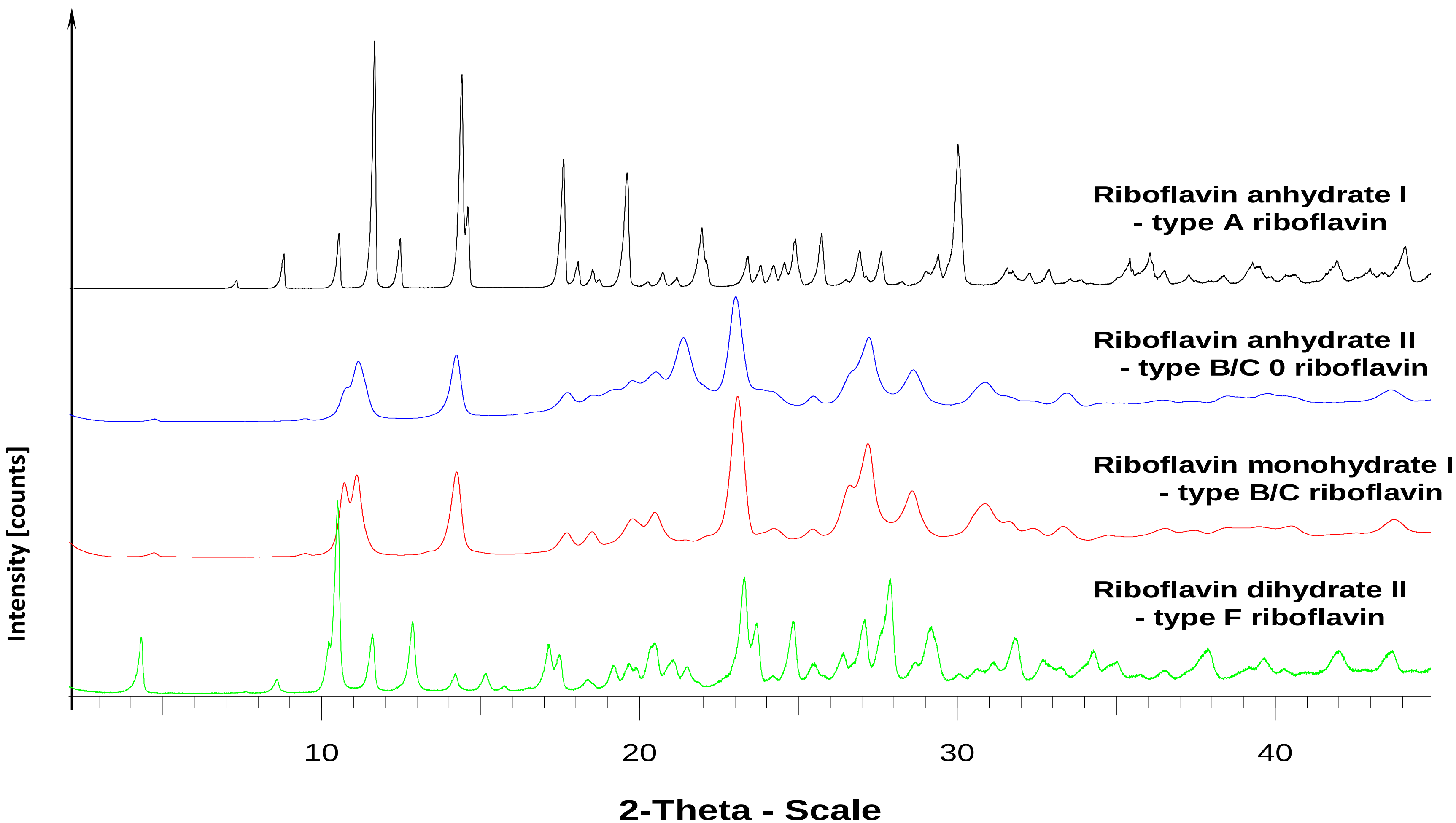
Mohnicke, M.: *On the control of riboflavin (vitamin B2) crystal structures*, Dissertation, Martin-Luther-Universität Halle-Wittenberg (2006), Aachen, Shaker Verlag 2007, ISBN: 978-3-8322-6064-4.

Microscope images of the crystals of form I (left) and form II (right)



Guo, J.: *Crystallization of Polymorphs: A Case Study on Astaxanthin and Apocarotenoic Ester,* Dissertation, Martin-Luther-Universität Halle-Wittenberg, 2009.

Powder XRD patterns of known riboflavin structures (part I)

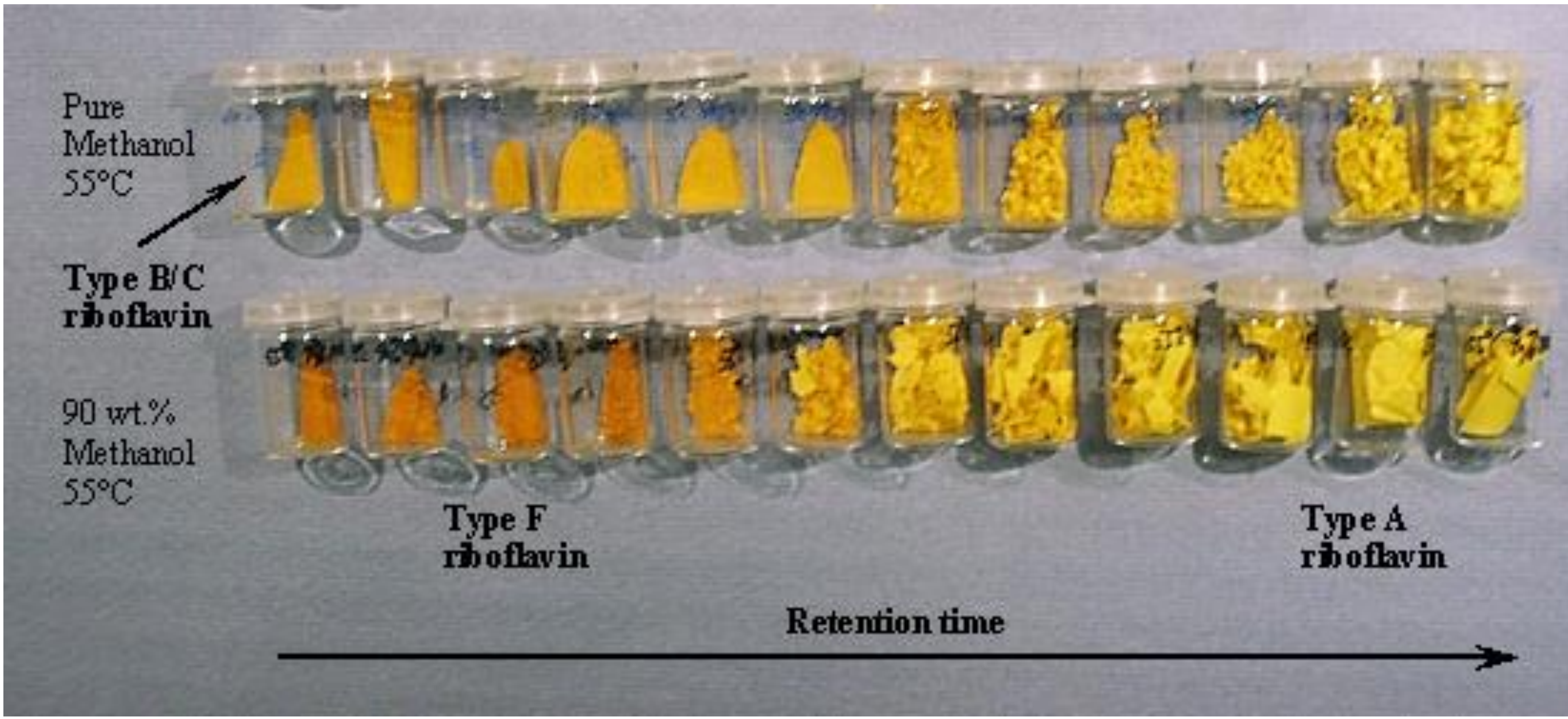


Mohnicke, M.: *On the control of riboflavin (vitamin B2) crystal structures*, Dissertation, Martin-Luther-Universität Halle-Wittenberg (2006), Aachen, Shaker Verlag 2007, ISBN: 978-3-8322-6064-4.

Example

polymorph

- Influence of crystal structure, temperature, retention time and solvent composition on the colour of riboflavin crystals]

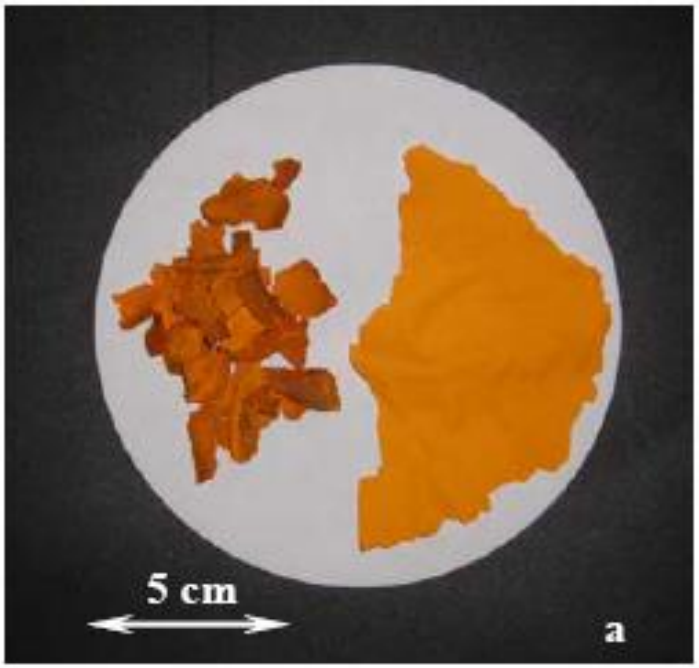


Mohnicke, M.: *On the control of riboflavin (vitamin B2) crystal structures*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, Shaker Verlag, Aachen, 2007.

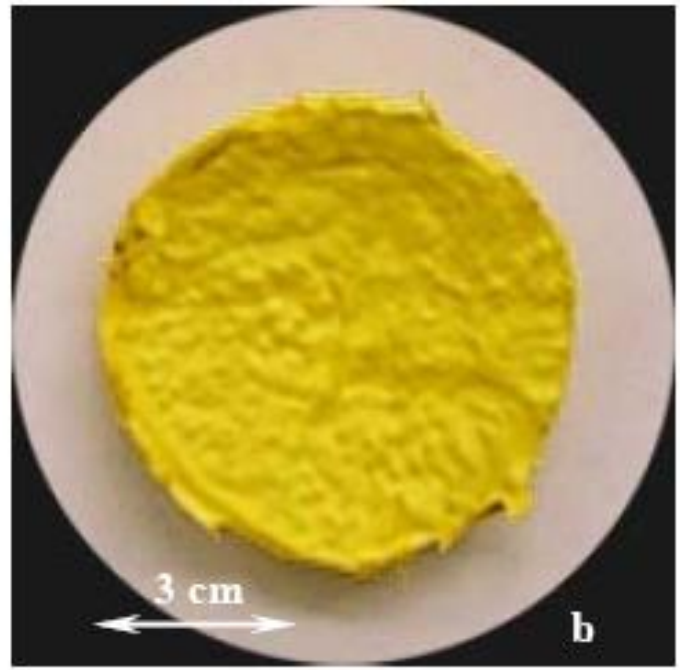
Example

polymorph

- Influence of solvent content on the crystal structure of riboflavin



type B/C riboflavin derived
from type E riboflavin

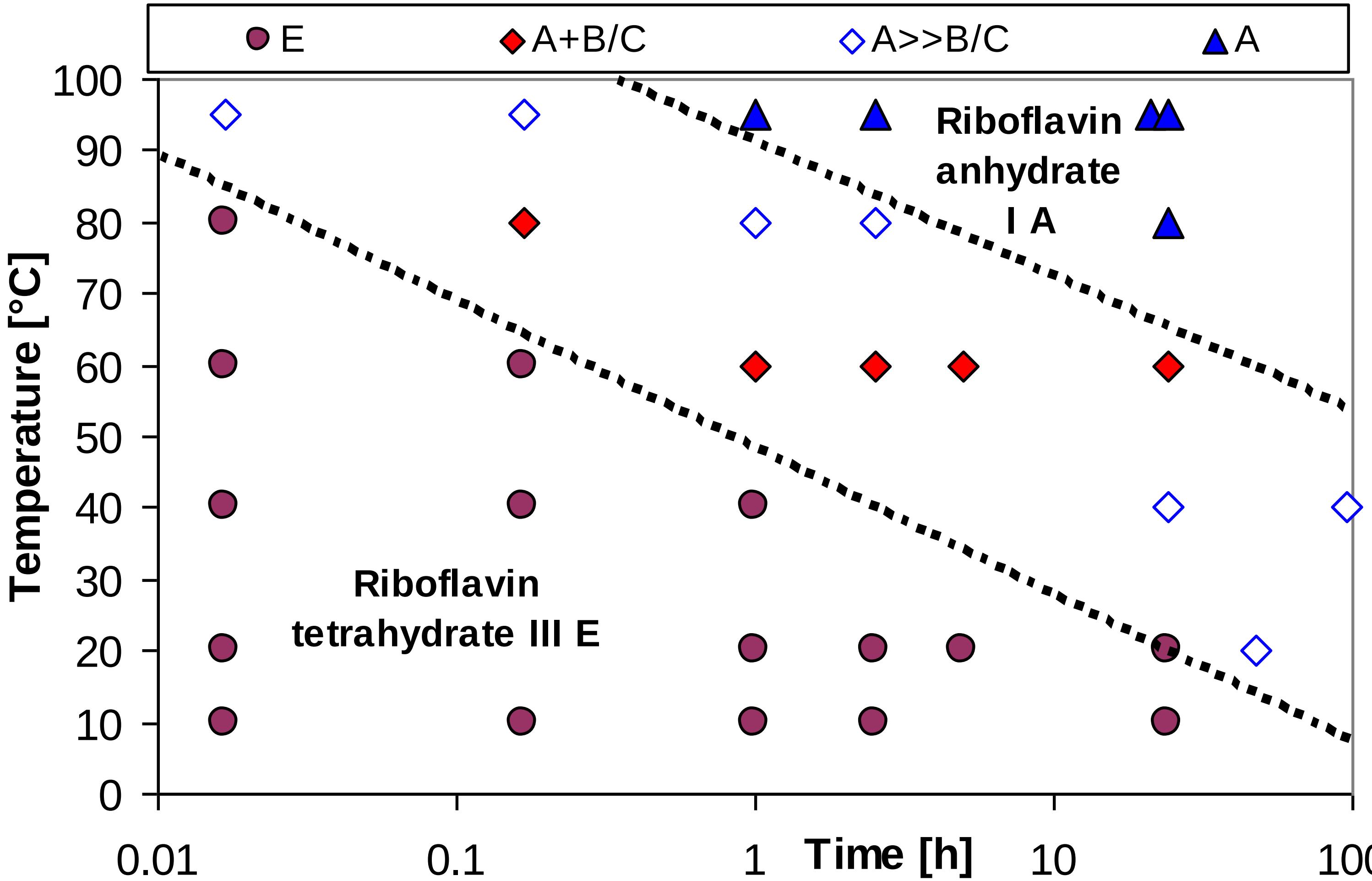


type A riboflavin derived
from type E riboflavin

Mohnicke, M.: *On the control of riboflavin (vitamin B2) crystal structures*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, Shaker Verlag, Aachen, 2007.

Example

- Phase transition behaviour of type E riboflavin in water

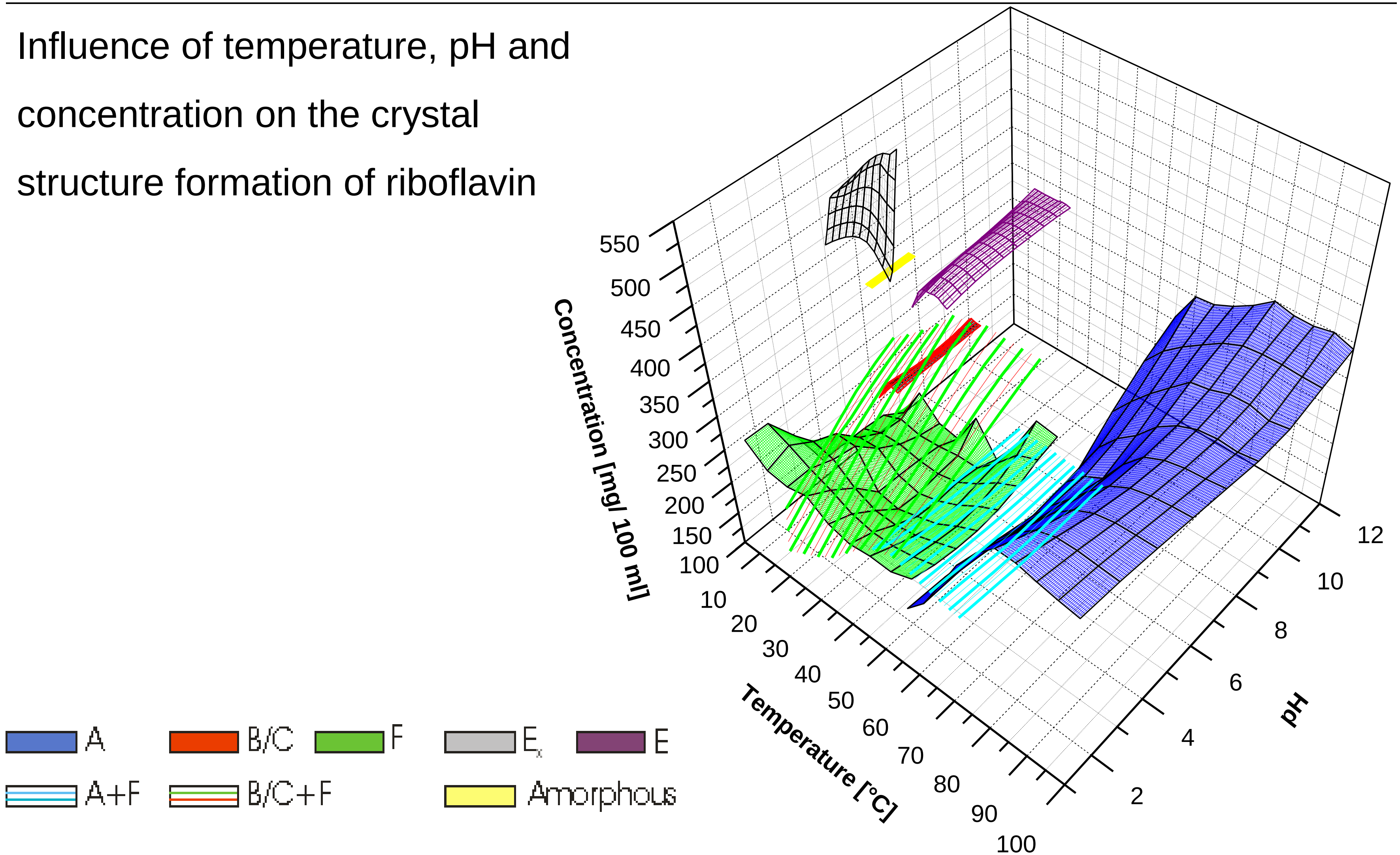


Mohnicke, M.: *On the control of riboflavin (vitamin B2) crystal structures*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, Shaker Verlag, Aachen, 2007.

Example

polymorph

- Influence of temperature, pH and concentration on the crystal structure formation of riboflavin

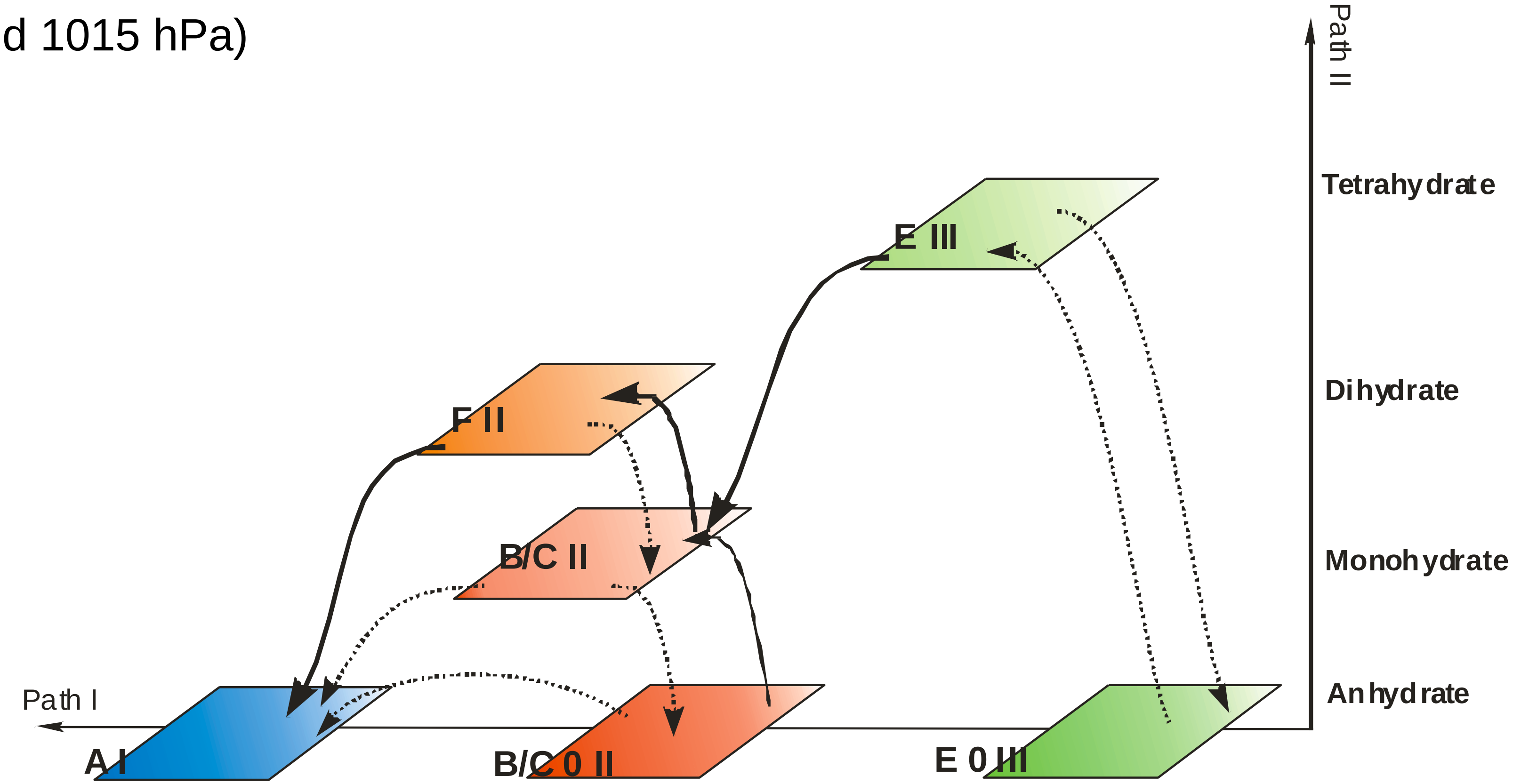


Mohnicke, M.: *On the control of riboflavin (vitamin B2) crystal structures*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, Shaker Verlag, Aachen, 2007.

Example

polymorph

- Phase transition pathways of riboflavin (Riboflavin anhydrate I (A)=thermodynamic stable crystal form of riboflavin, at room temperature, normal pressure (18 to 25 °C and 1015 hPa)



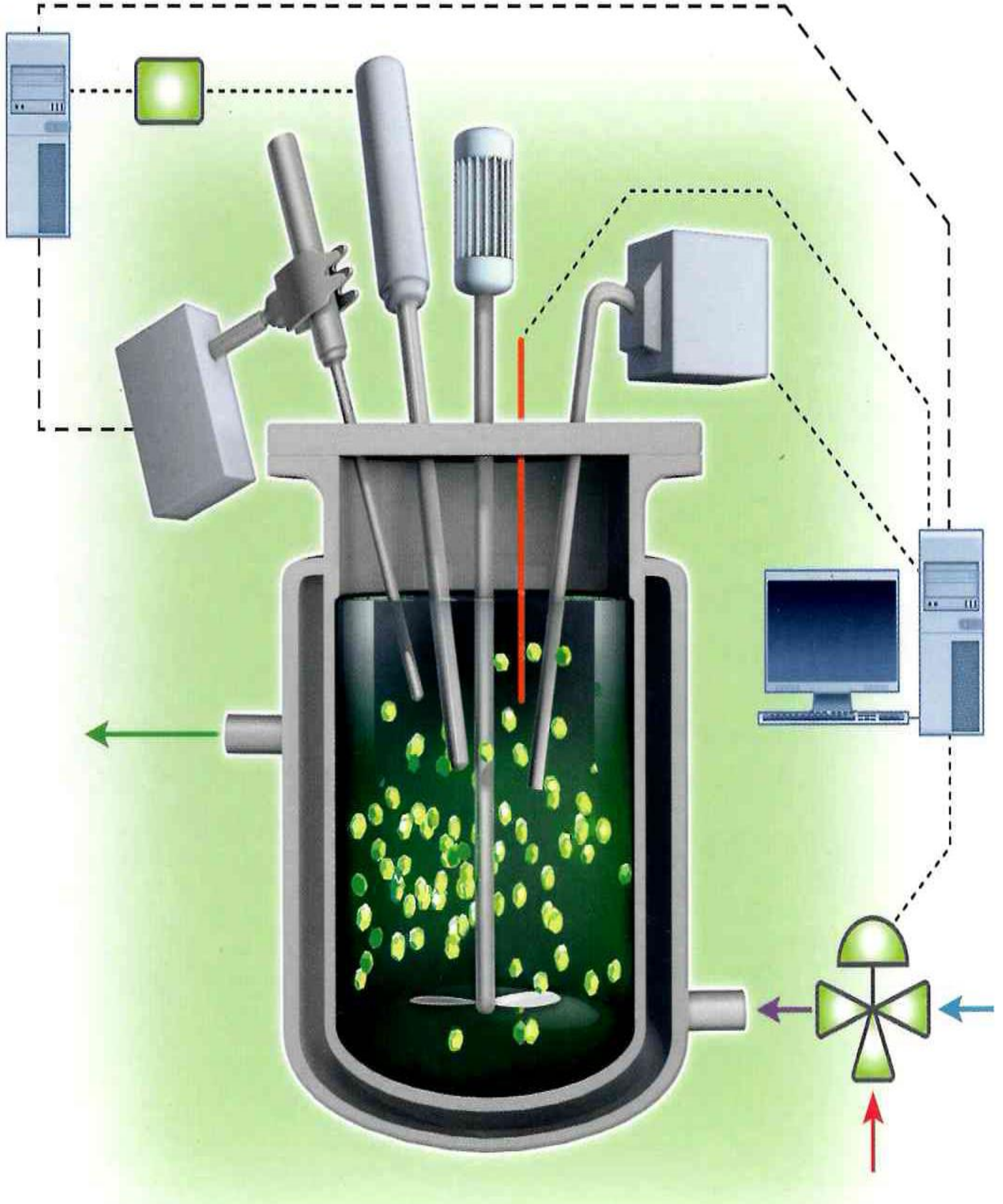
Mohnicke, M.: *On the control of riboflavin (vitamin B2) crystal structures*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, Shaker Verlag, Aachen, 2007.

Thank you for your attention!

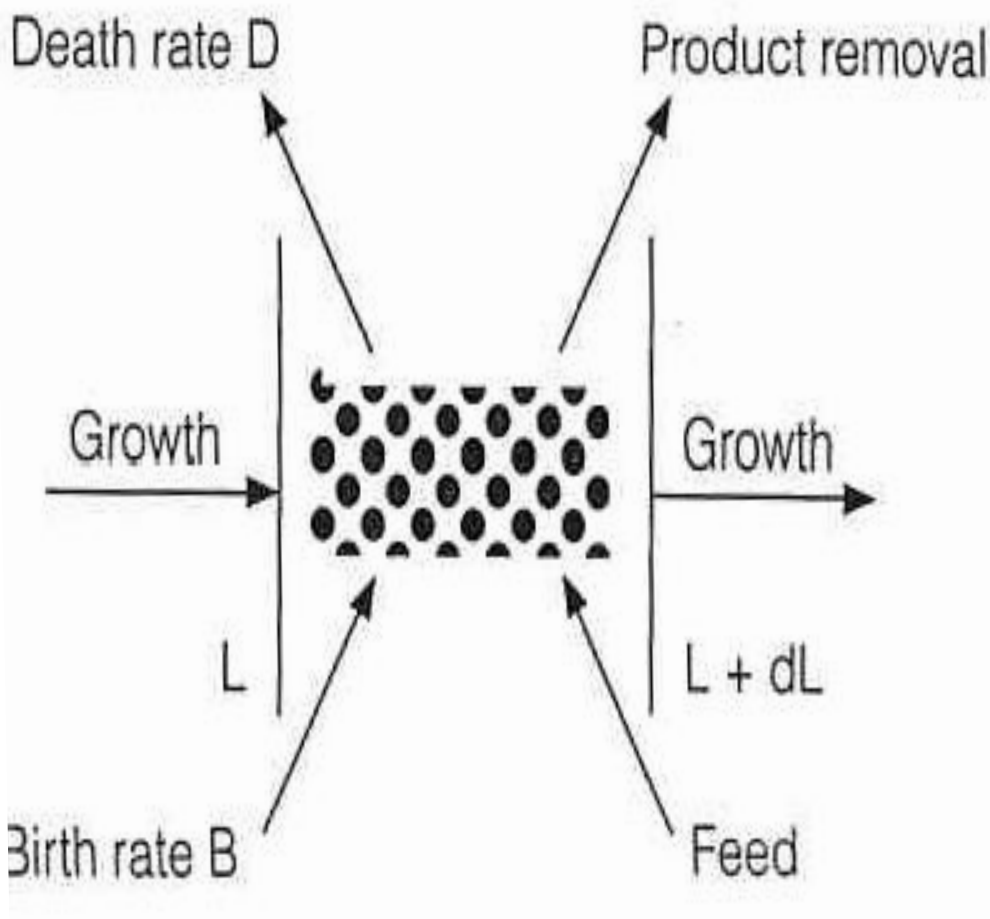
Martin-Luther-Universität Halle-Wittenberg
Zentrum für Ingenieurwissenschaften,
Verfahrenstechnik / TVT

Process control in batch crystallization

Prof. Dr.-Ing. habil. Joachim Ulrich



$$\frac{\partial n}{\partial t}+\frac{\partial(Gn)}{\partial L}+n\frac{\partial V}{V\partial t}+D(L)-B(L)+\sum_k\frac{\dot{V}_i n_i}{V}=0$$



$$\frac{\partial(Gn)}{\partial L} + \sum_k \frac{n_i \dot{V}_i}{V} = 0$$

$$\frac{\partial(Gn)}{\partial L} + n\frac{\dot{V}}{V} = 0$$

$$\frac{\partial(Gn)}{\partial L} + \frac{n}{\tau} = 0$$

$$G\frac{\mathrm{d}n}{\mathrm{d}L}+\frac{n}{\tau}=0$$

$$n = n_0 \exp\left(-\frac{L}{G\tau}\right)$$

$$\ln\left(\frac{n}{n_0}\right) = -\frac{L}{G\tau}$$

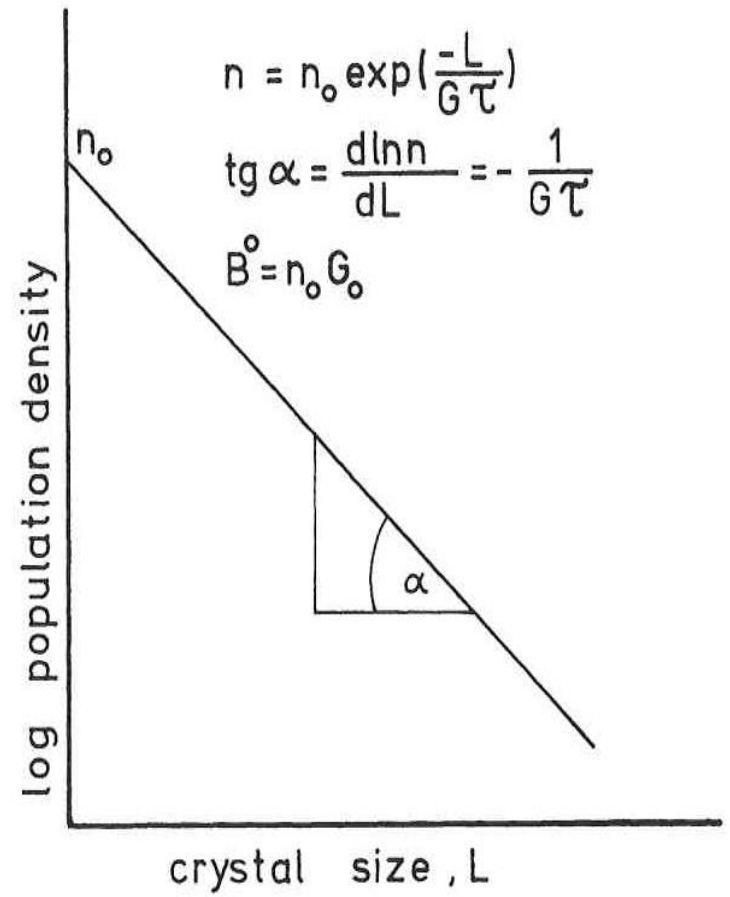
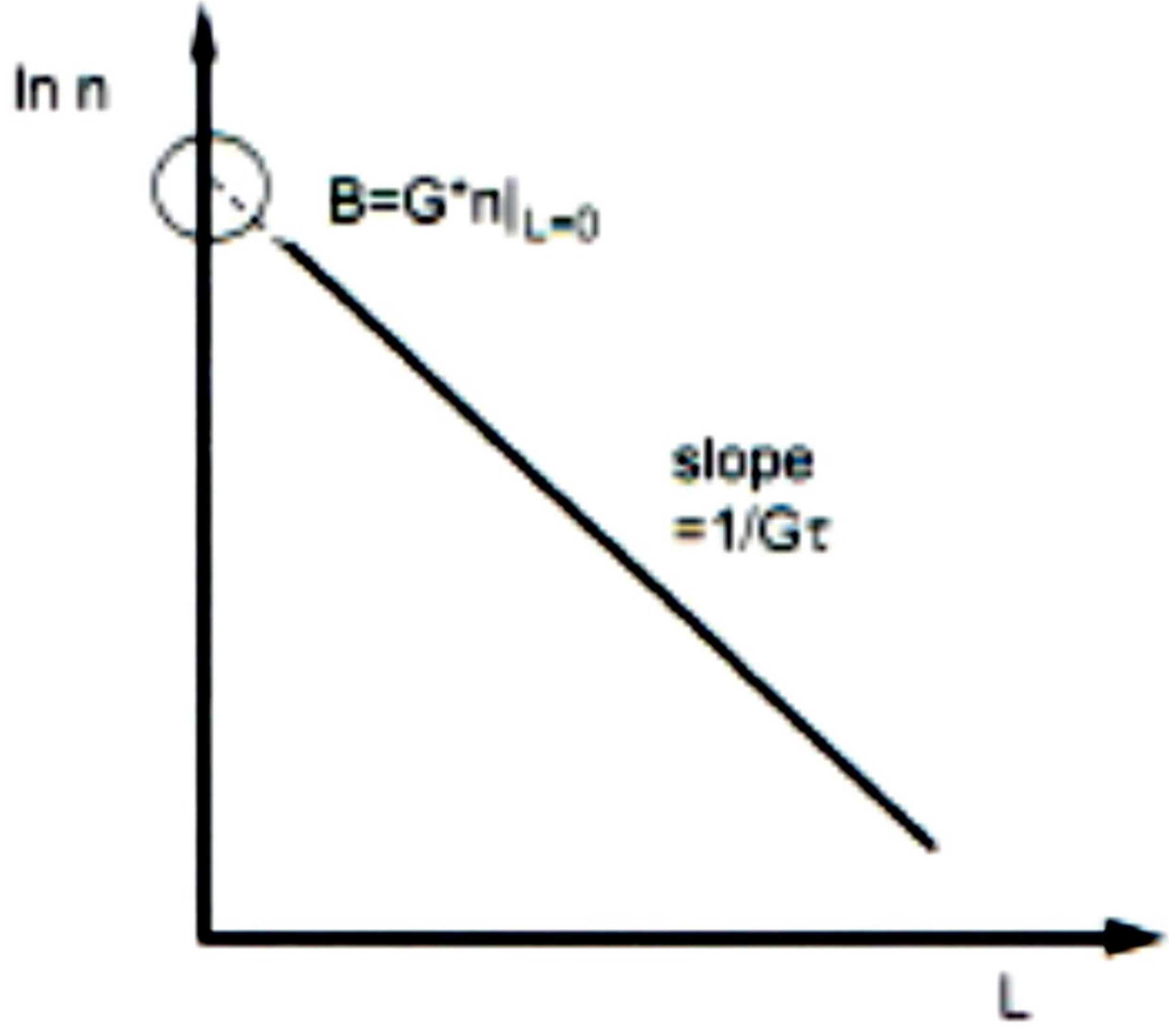
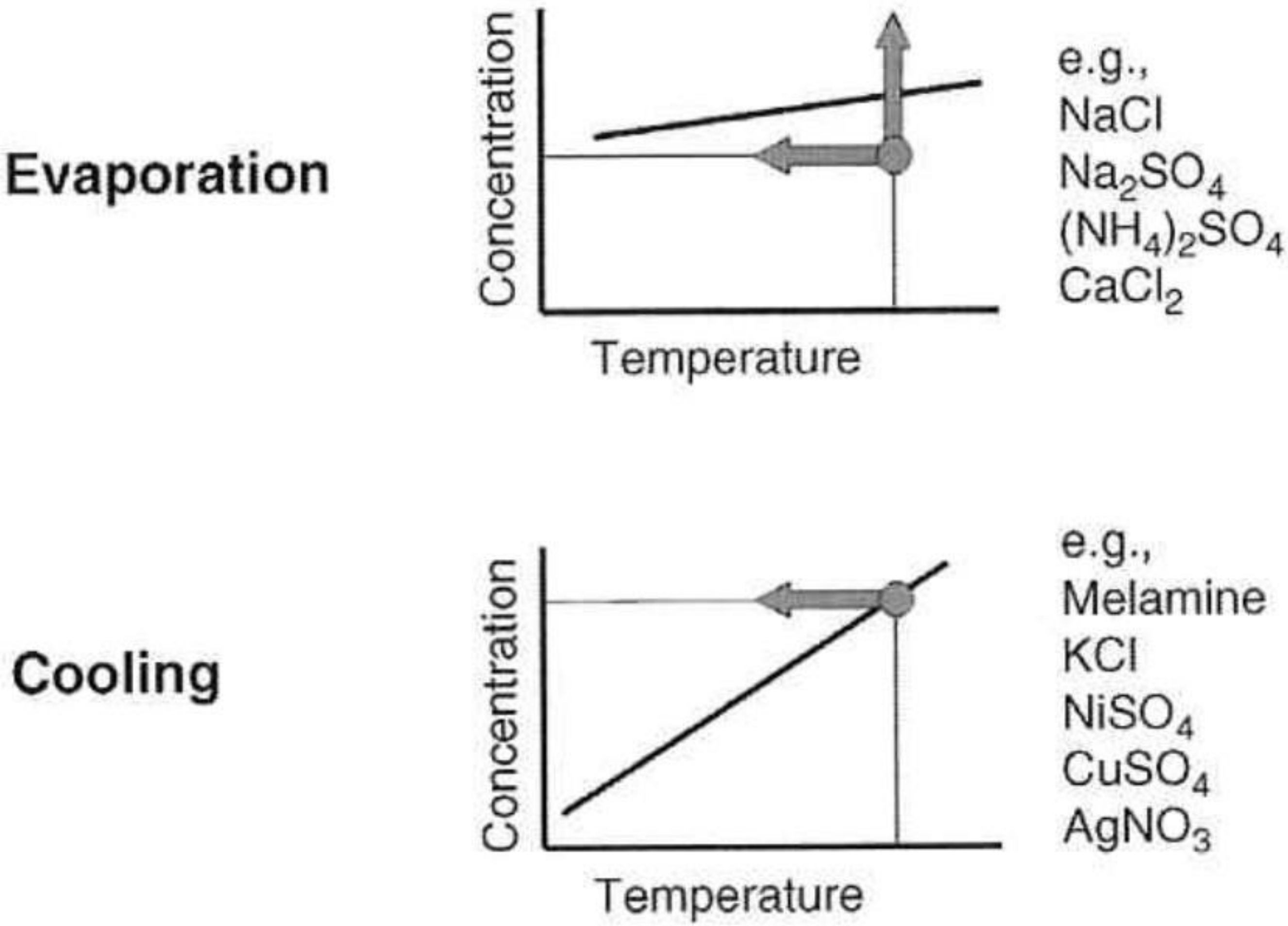
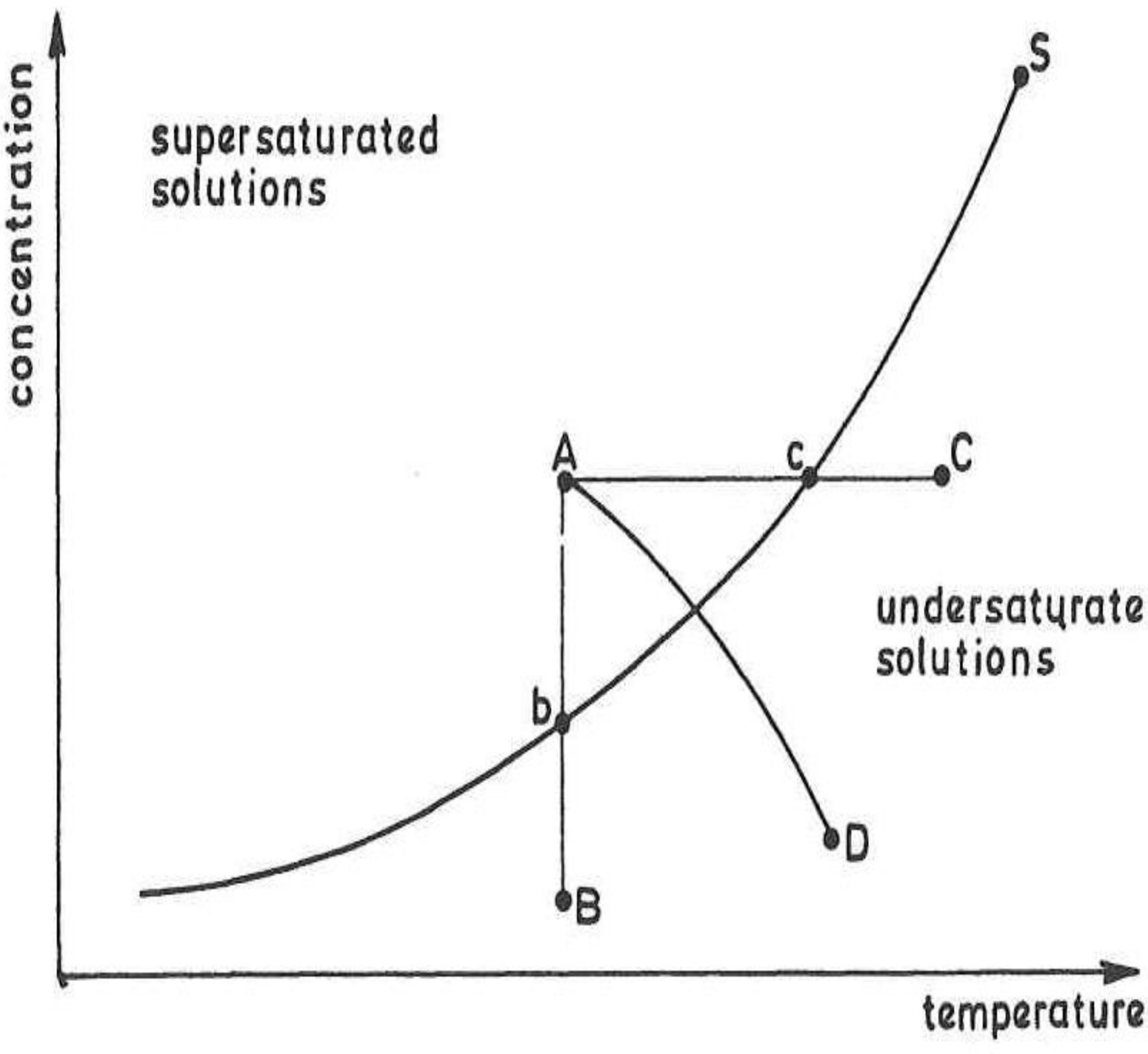


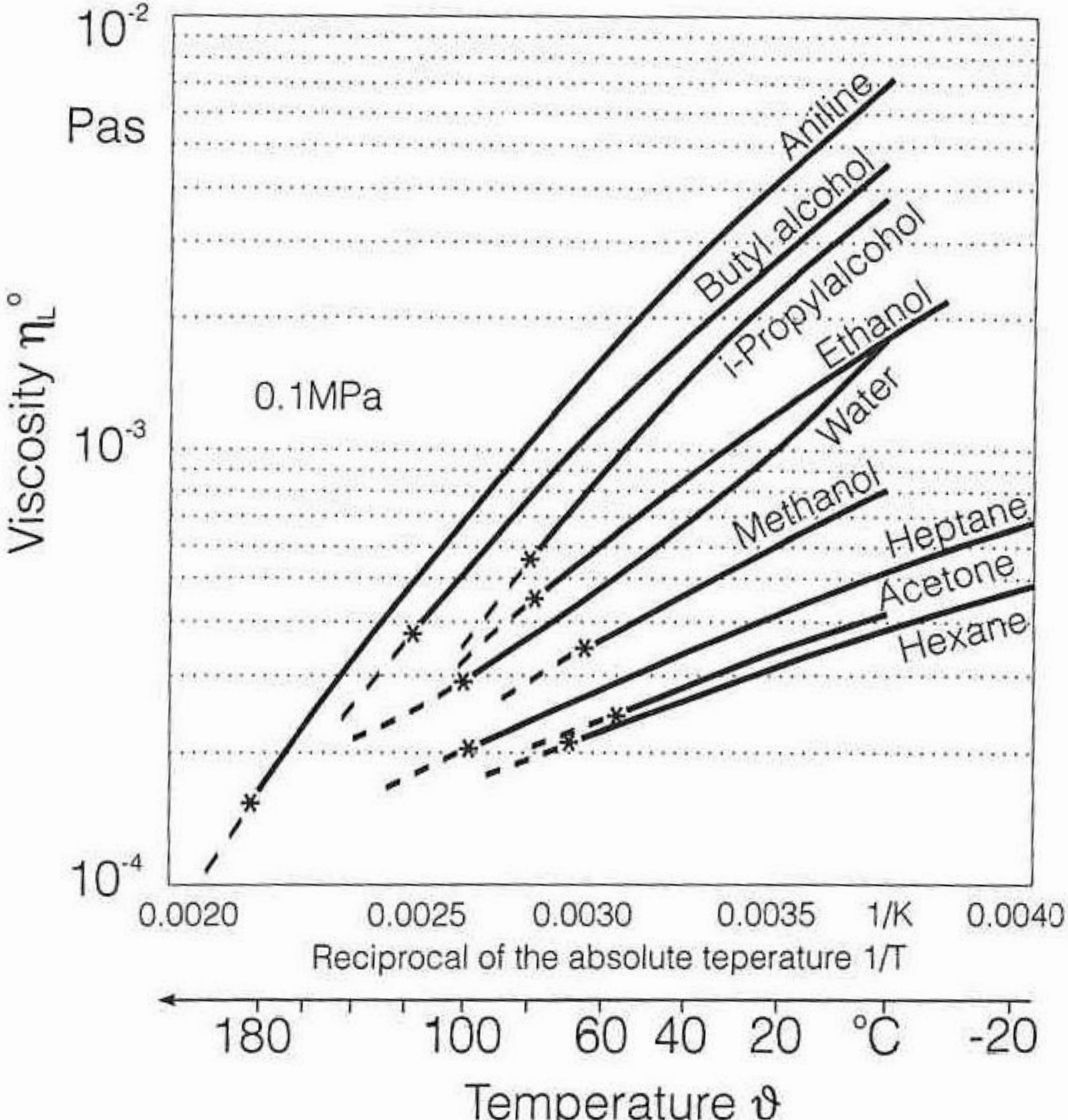
Figure 7.2 Theoretical population density plot for a steady state MSMPR crystallizer operating at the following conditions:

- no secondary nucleation other than at zero size
- no attrition other than at zero size
- no crystal breakage
- no agglomeration
- no growth dispersion
- crystal growth rate is independent of size.


ln n
B=G*n|L=0
slope
=1/Gτ
L

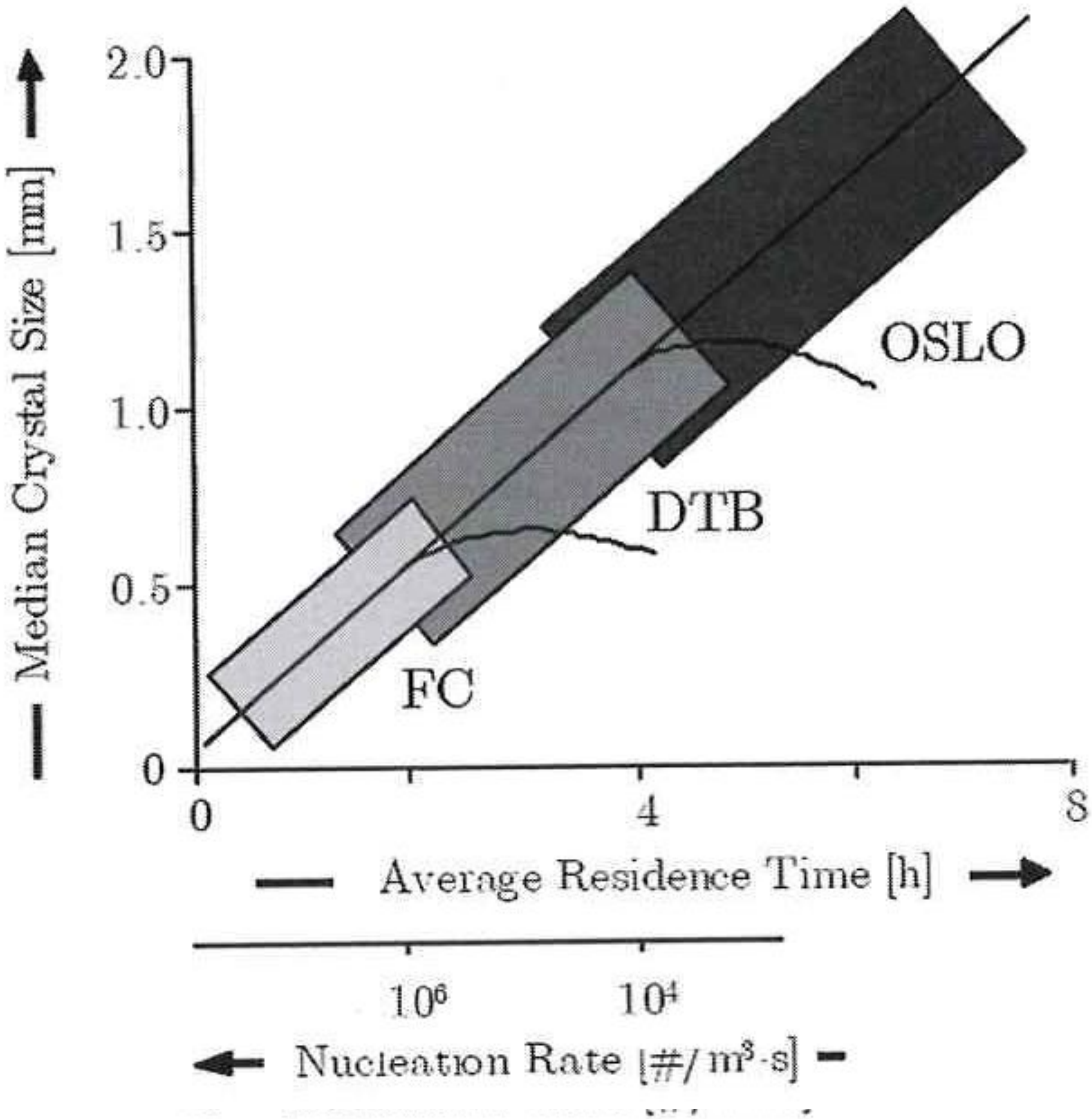

Evaporation
Concentration
Temperature
e.g.,
NaCl
Na_2SO_4
$(NH_4)_2SO_4$
$CaCl_2$
Cooling
Concentration
Temperature
e.g.,
Melamine
KCl
$NiSO_4$
$CuSO_4$
$AgNO_3$

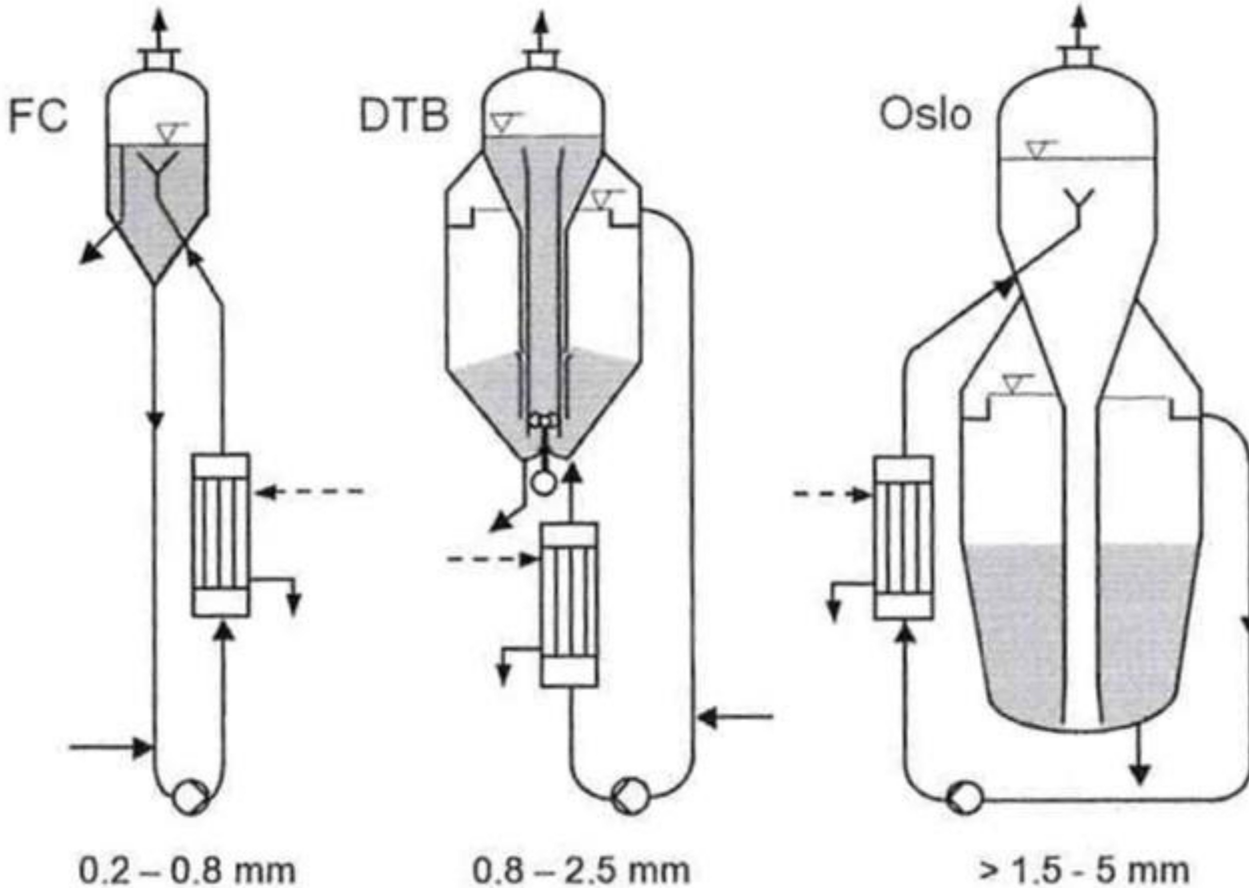

concentration
supersaturated
solutions
S
A
c
C
undersaturated
solutions
b
D
B
temperature



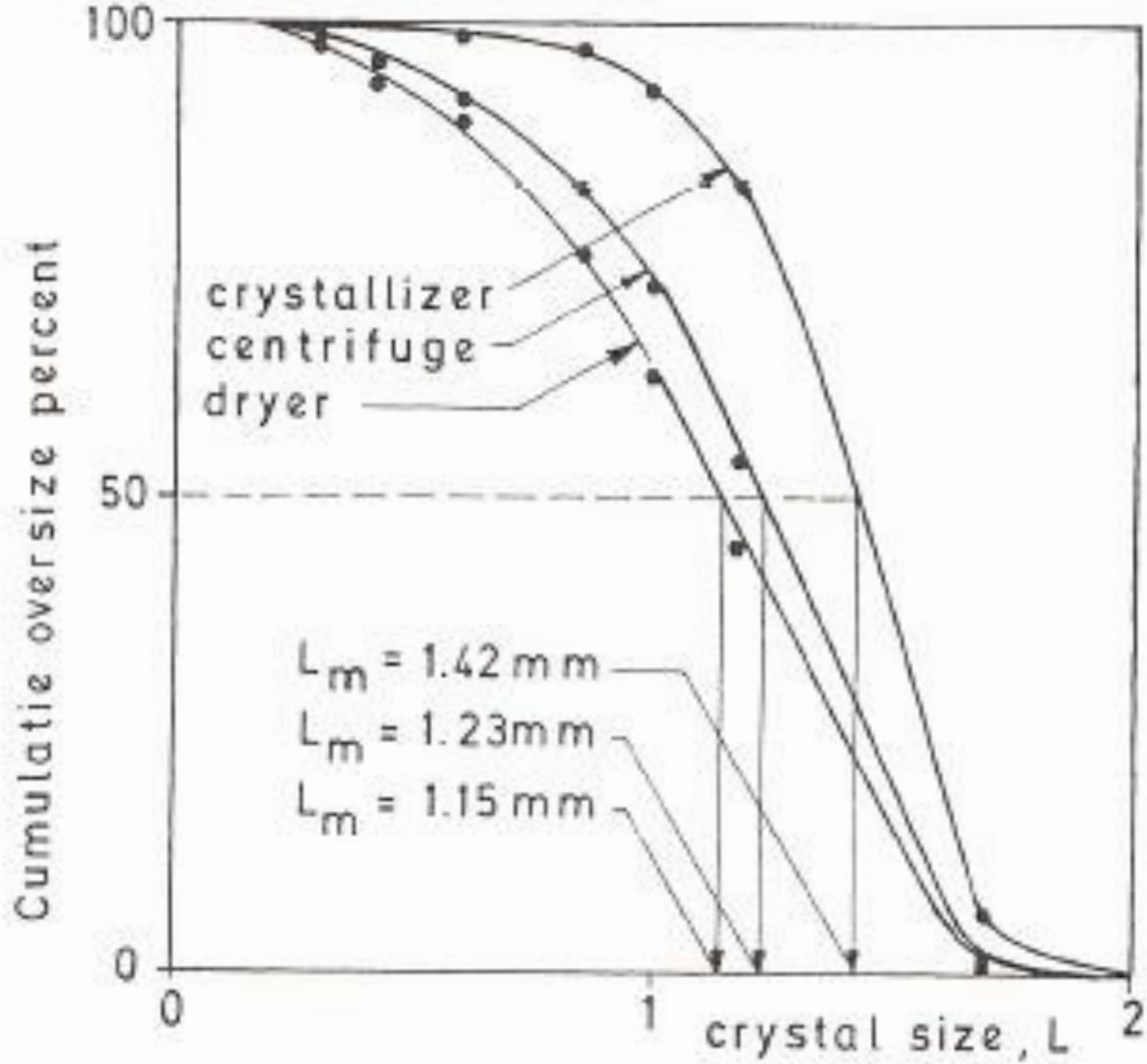
10^-2
Pas
Viscosity η_L°
0.1MPa
10^-3
10^-4
Aniline
Butyl alcohol
i-Propylalcohol
Ethanol
Water
Methanol
Heptane
Acetone
Hexane
0.0020
0.0025
0.0030
0.0035
1/K
0.0040
Reciprocal of the absolute teperature 1/T
180
100
60
40
20
°C
-20
Temperature ϑ

FC crystallizers:	0.2-0.8 mm
DTB crystallizers:	0.8-2.5 mm
Oslo crystallizers:	1.5-5 mm


2.0
1.5
1.0
0.5
0
Median Crystal Size [mm]
OSLO
DTB
FC
0
4
8
Average Residence Time [h]
10^6
10^4
Nucleation Rate [#/m³·s]



(a)
(b)
(c)



100
50
0
Cumulatie oversize percent
crystallizer
centrifuge
dryer
L_m = 1.42 mm
L_m = 1.23mm
L_m = 1.15 mm
0
1
2
crystal size , L

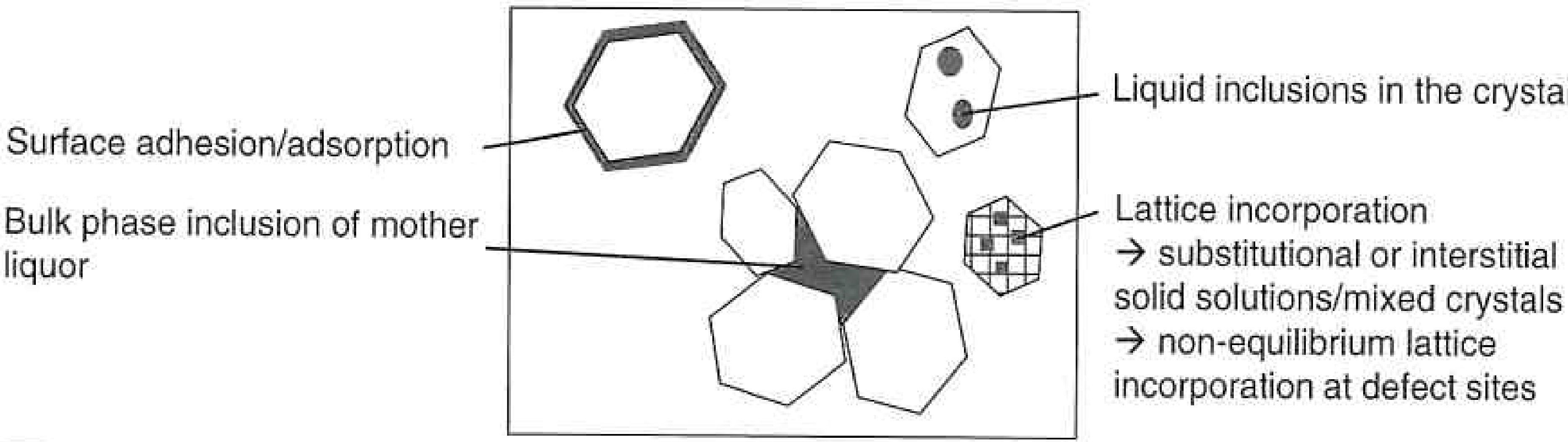
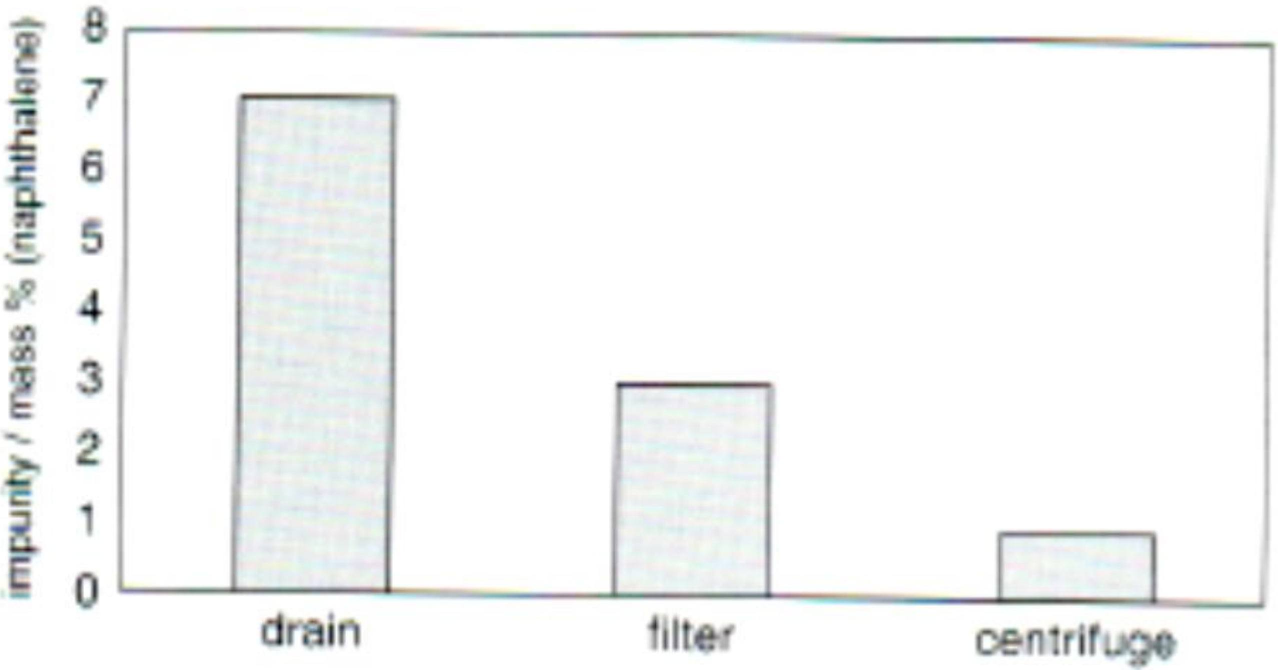
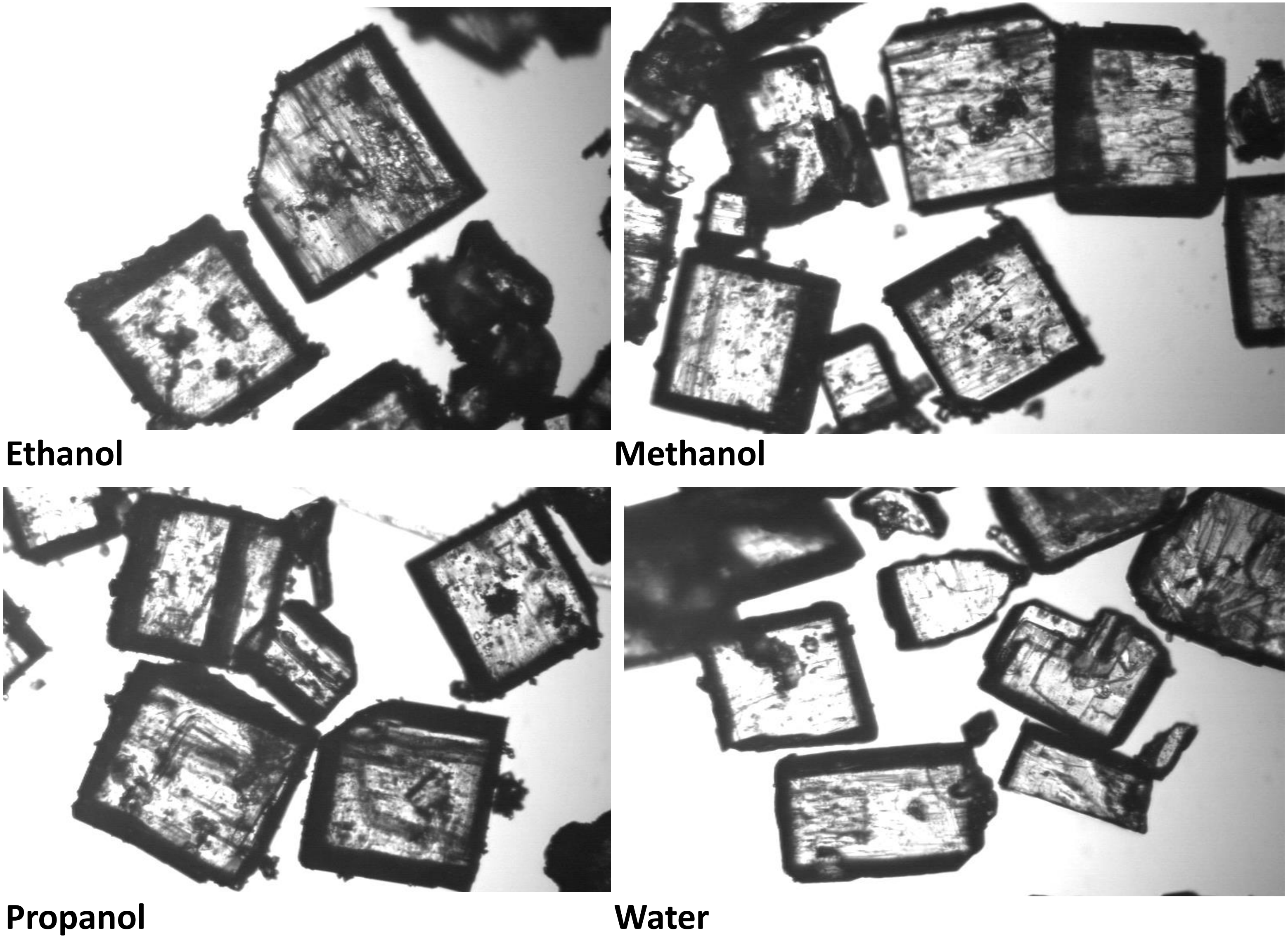


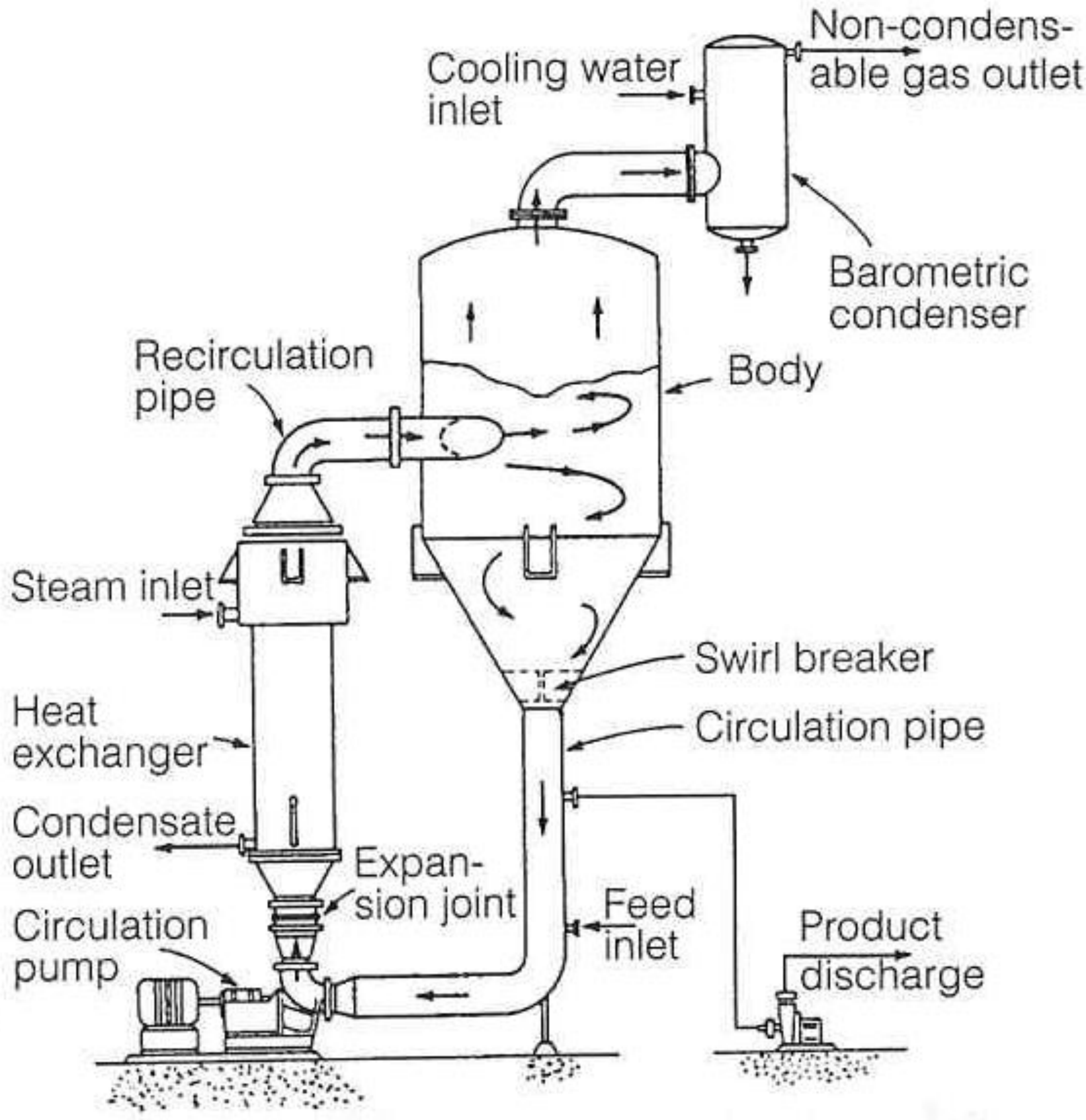
Figure 7.1 Location of impurities in a crystalline product after solid–liquid separation. The growing crystal itself can incorporate impurities either in the lattice on a molecular level or as three-dimensional faults, liquid inclusions (right). In addition, the crop can contain impurities via mother liquor, either adsorbed on the crystals or entrapped between crystals (left).

	Residual moisture content related to the solid (wt%)
Sedimentation	20–40
Filtration	8–18
Centrifugation	4–8

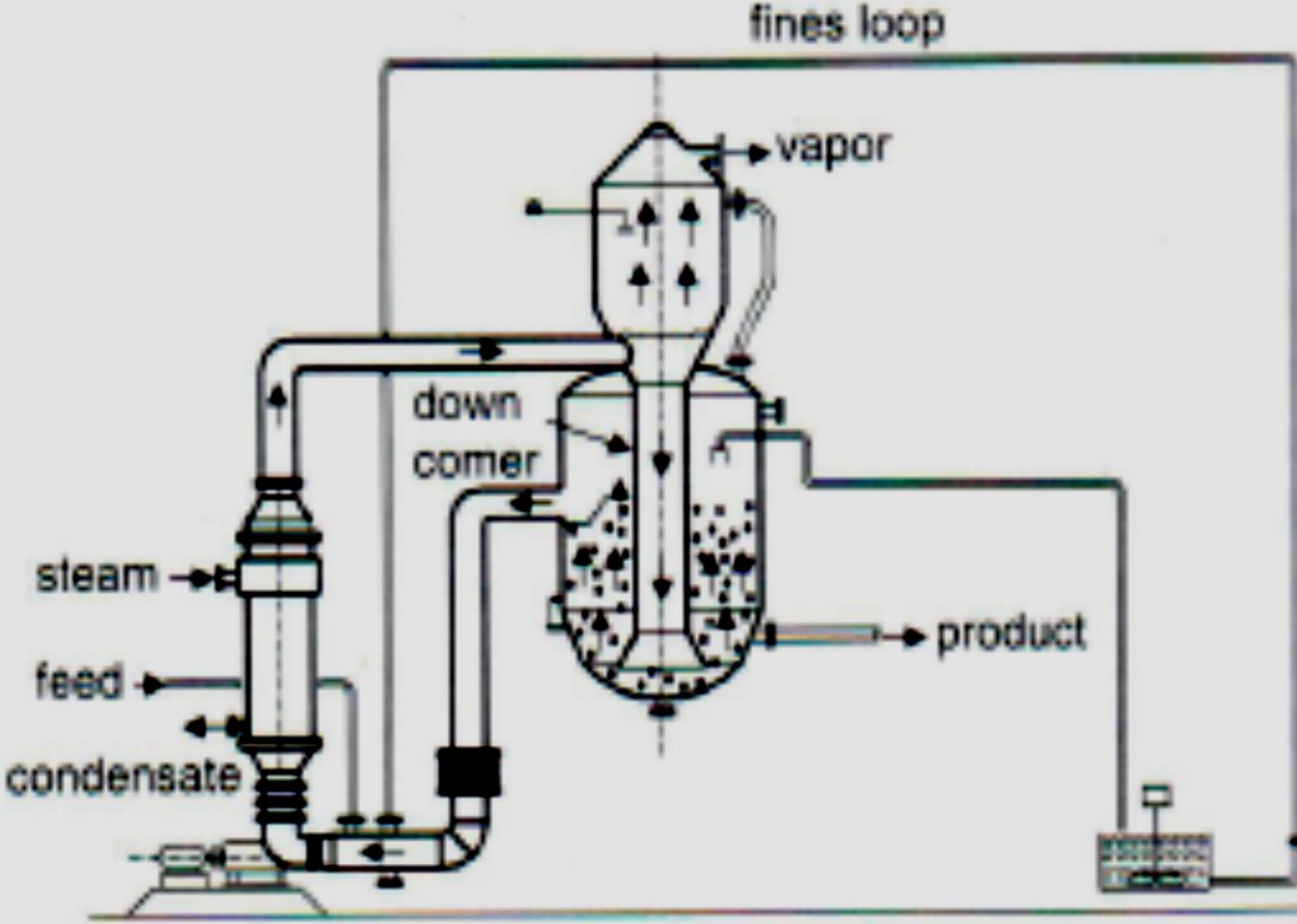


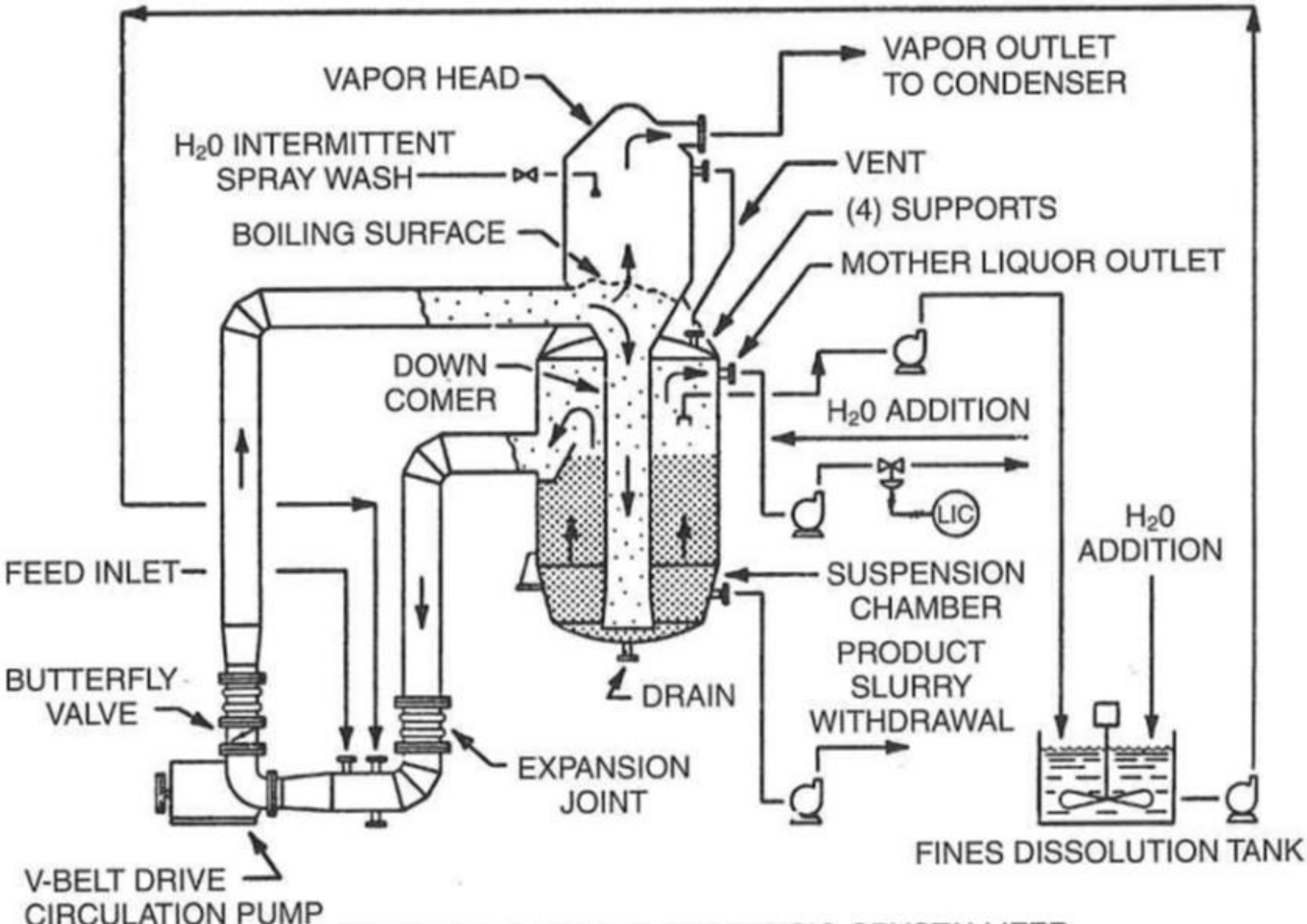


Omar, W., Mohnicke, M., Ulrich, J.: *Influence of solvents on the crystal growth kinetics of ascorbic acid,* in WASIC 2003, Workshop on Advance in Sensoring in Industrial Crystallization, edited by N. Bulutcu, H. Gürbüz **&** J. Ulrich, Istanbul Technical University, Istanbul, Turkey 2003, 113-120, (ISBN: 975-561-254-8).



Non-condens-
able gas outlet
Cooling water
inlet
Barometric
condenser
Recirculation
pipe
Body
Steam inlet
Swirl breaker
Heat
exchanger
Circulation pipe
Condensate
outlet
Expan-
sion joint
Feed
inlet
Circulation
pump
Product
discharge


fines loop
vapor
down
comer
steam
product
feed
condensate



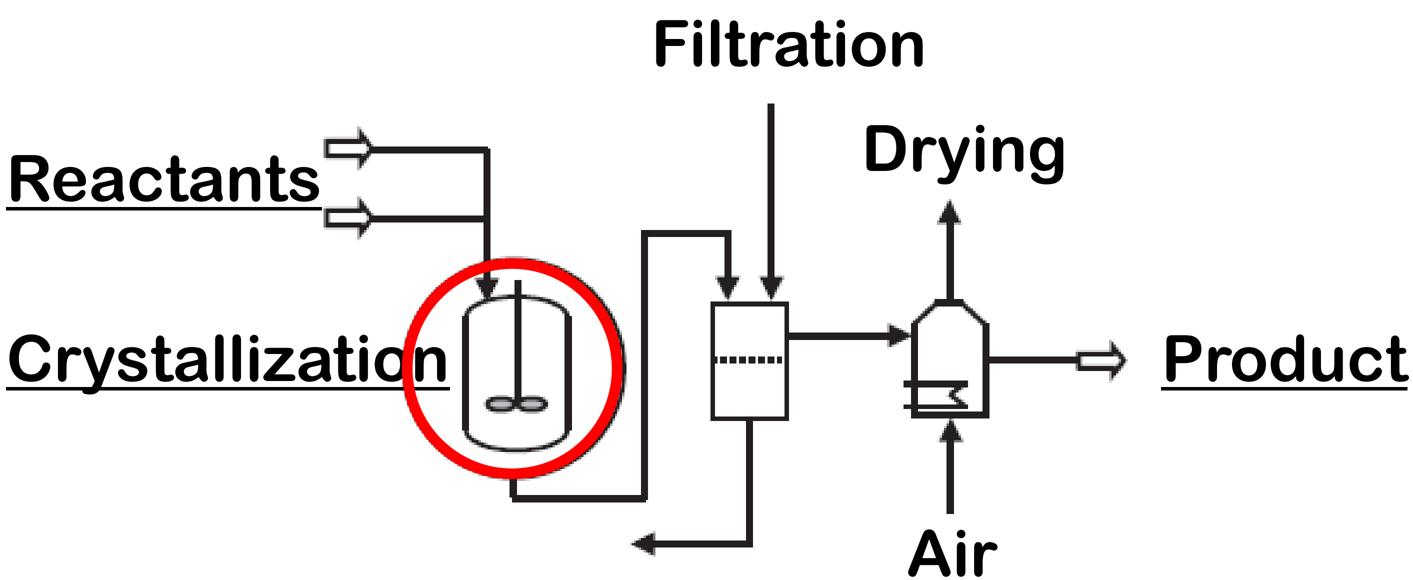
SWENSON FLUIDIZED SUSPENSIO CRYSTALLIZER

$$\Delta C = 0.4 - 0.6 \ \Delta C_{met}$$

m_T = 10 – 30 mt% crystals in suspension

k_G = f(T, …)   Target: T high

Crystallization Process



Aims: Product *quality* & *quantity*

- purity
- morphology + CSD
- flowability, shelf life,…

- maximum yield
- efficiency (materials, energy…)

Thermodynamically stable phases of organic and inorganic substances depend on:

- Temperature
- Pressure
- surrounding media:
  - air (relative humidity)
  - solvent (solubility)
- additional: mechanical stress (e.g. grinding)

→ **A change of these parameters often leads to metastable modifications**

Yield

- mass growth rates decrease
  → decreasing yield
- loss of charges

- mass growth rates can increase?
- expanded metastable zone width
  → higher supersaturations?
- prevent scaling?

Product Quality

- crystal habit changes
  → filtration, transport and storage problems
  → useless product
- undesired crystal size distribution

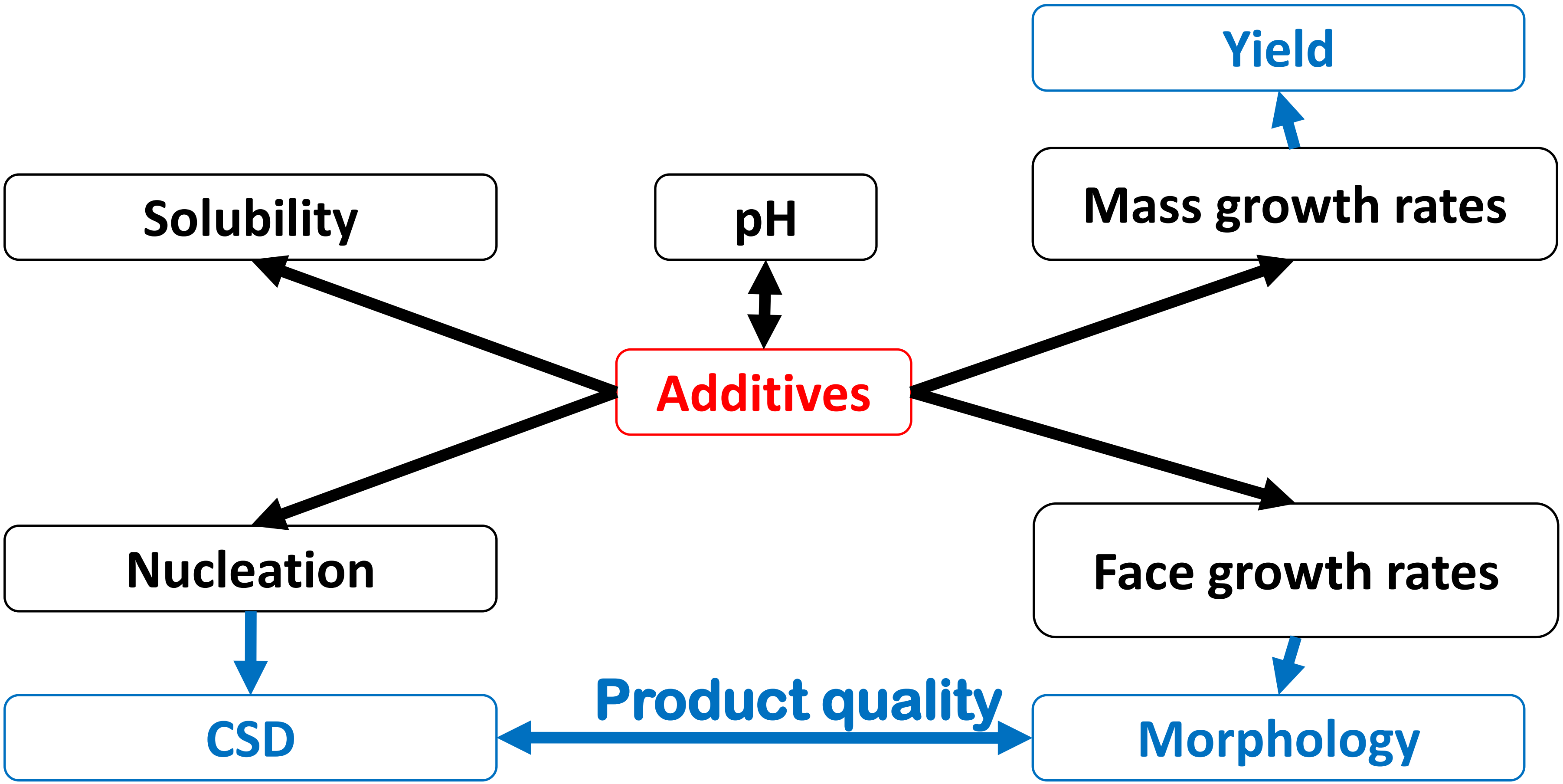
- directed crystal modifying
  → special applications
- “tailor-made”-additives

Aim of crystallization processes

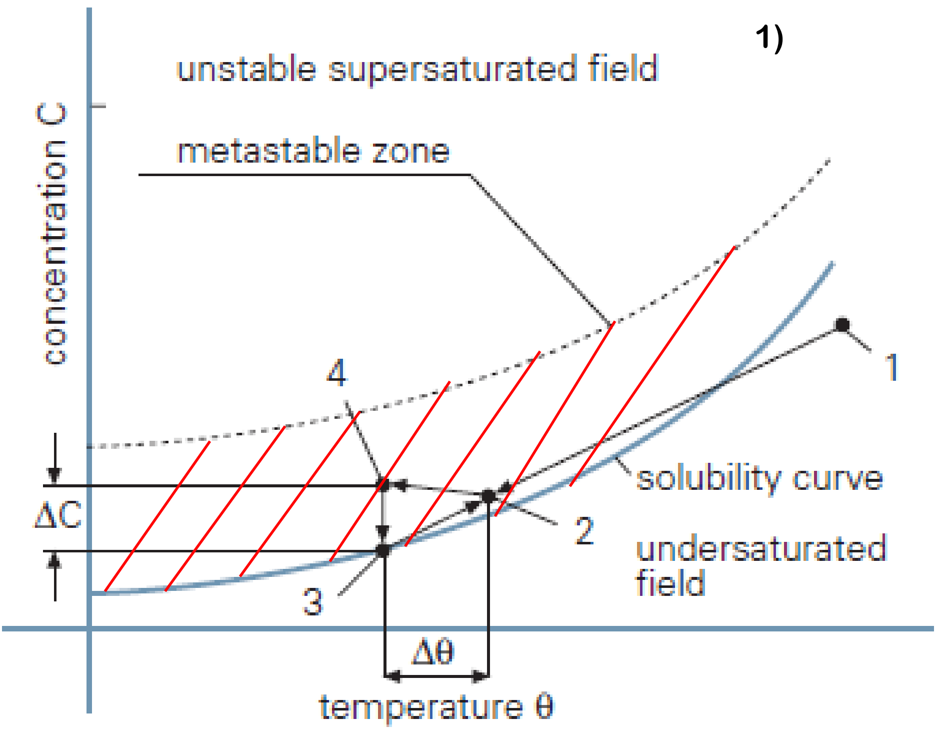
The crystallization process includes the determination of:

- Purification
- Particle size (distribution)
- Modification (polymorph / solvates)
- Shape design
- Lower costs (reduction of number of steps)

Effects of additives – An Overview



Crystallization Process – A closer look



Metastable zone width (MZW):

- zone for industrial crystallizations
- no primary homogeneous nucleation
- secondary nucleation → control of CSD

Supersaturation **σ *„driving force“***

- σ should not exceed MZW
- maximized for maximum growth rate

Where can additives affect the crystallization process?

Tools in crystallization

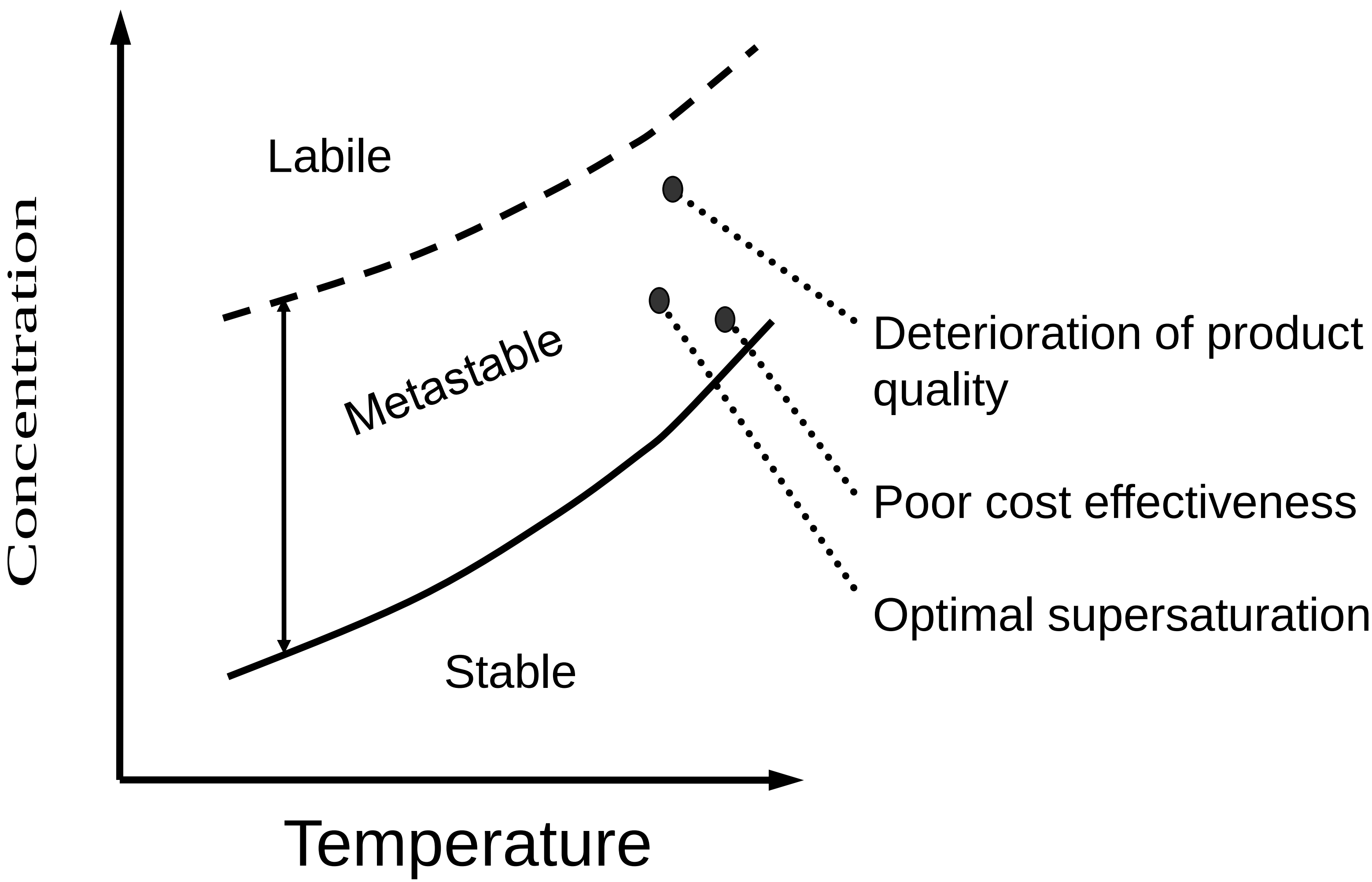
❖ **Thermodynamics:**

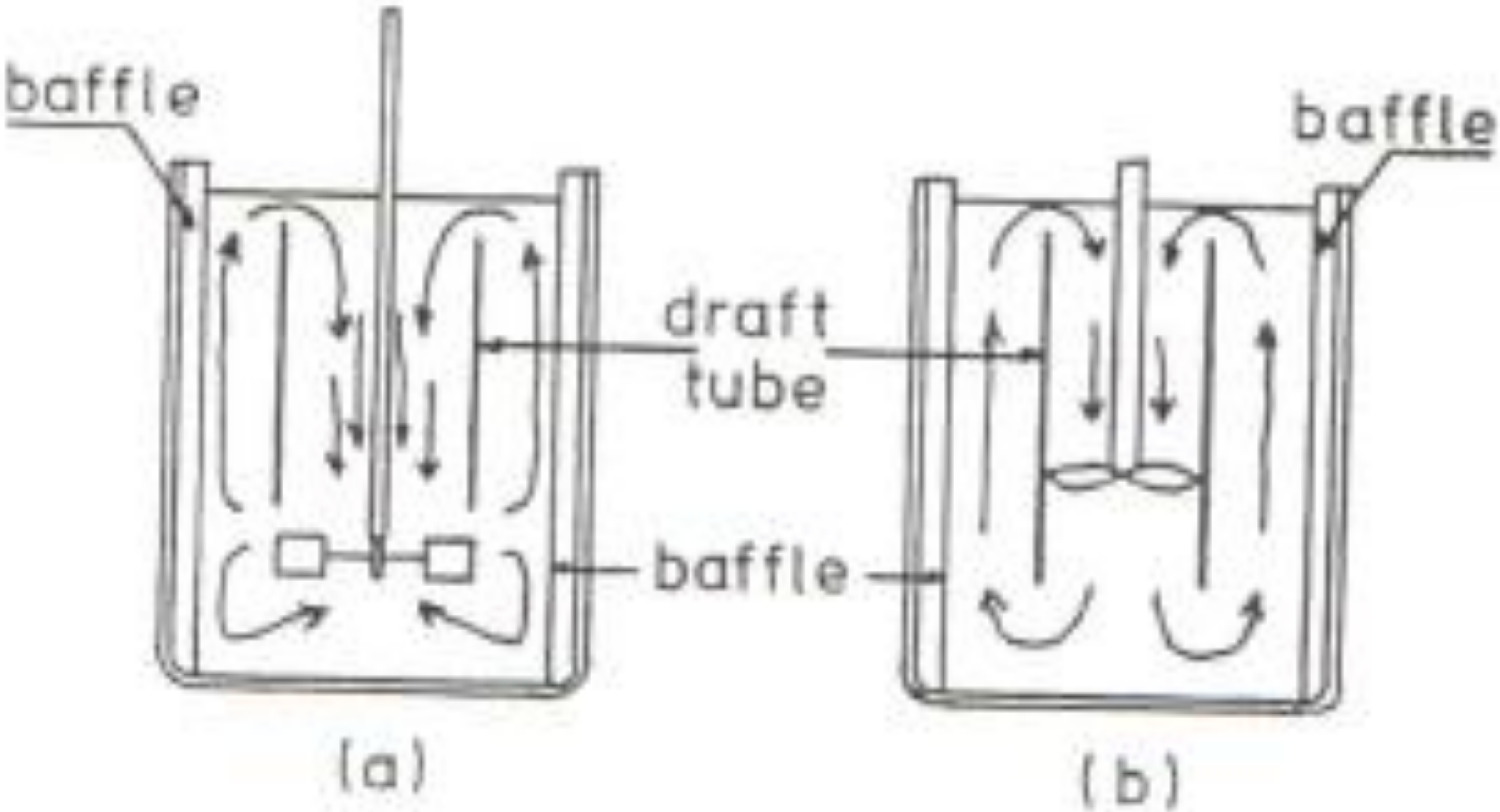
- Driving force (program)
- Phase diagram

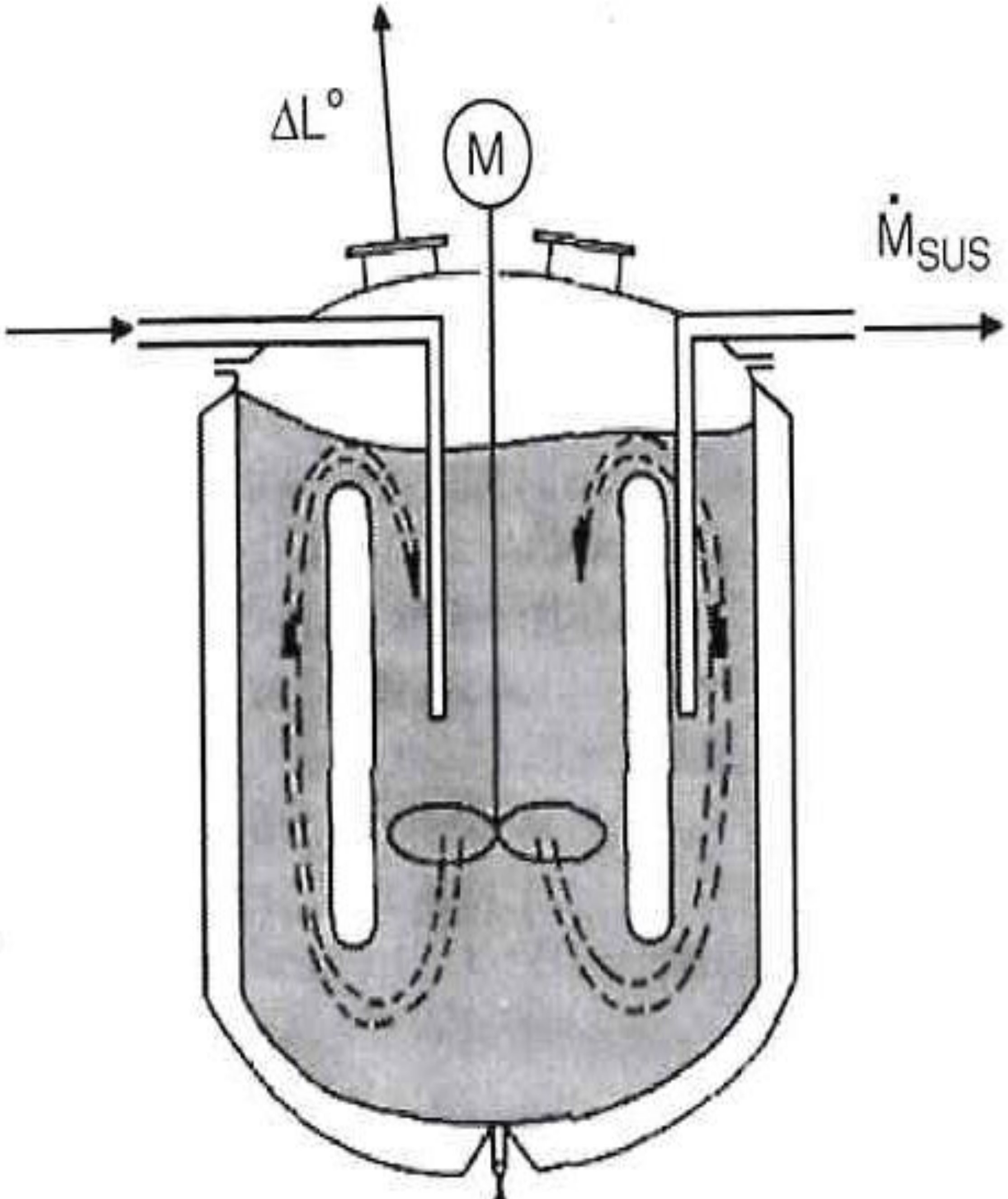
❖ **Kinetics:**

- Nucleation (place, how, when)
- Retention time
- Impurities

Metastable zone




baffle
baffle
draft
tube
baffle
(a)
(b)


ΔL°
M
$\dot{M}_{SUS}$

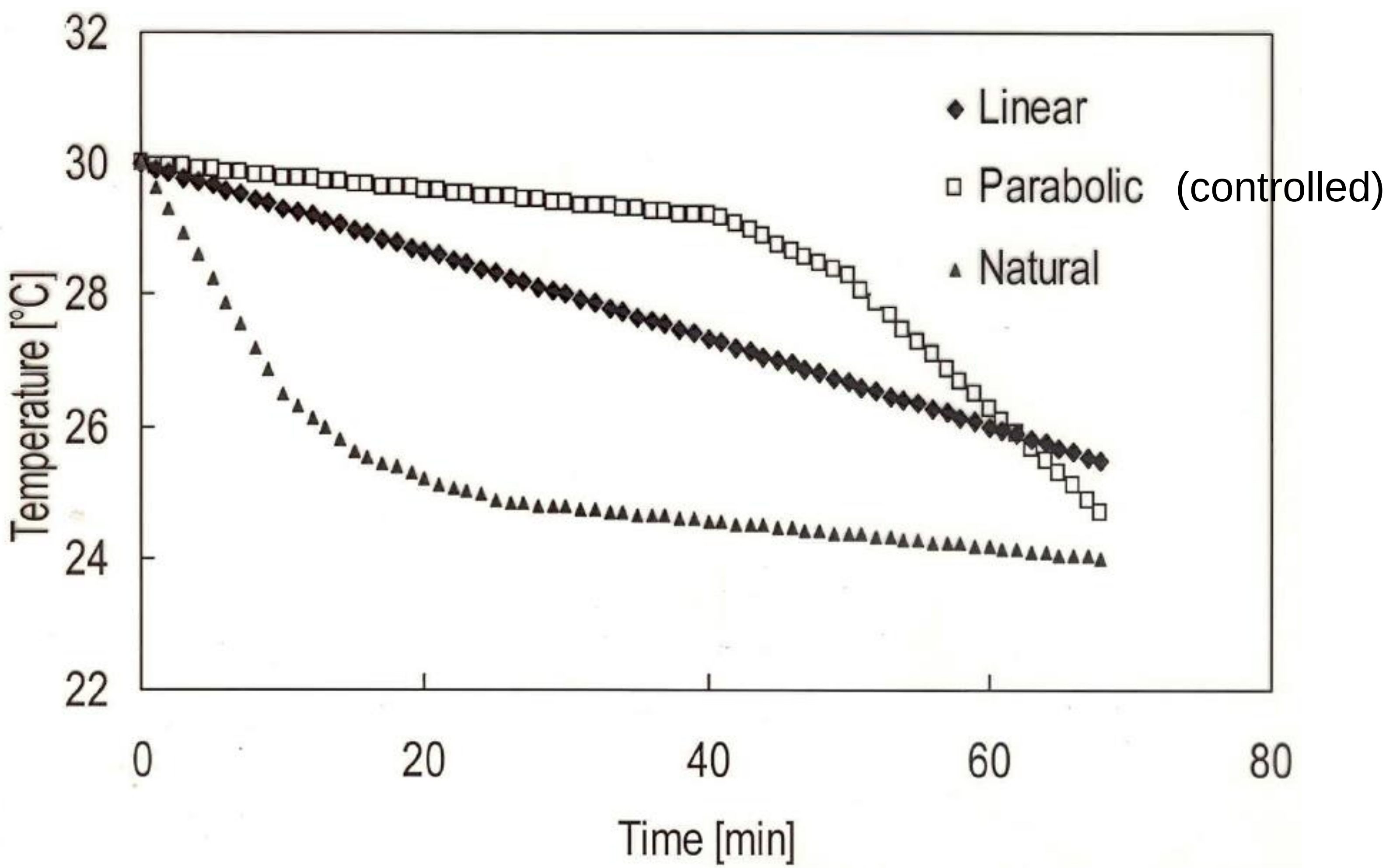
Batch crystallization

- How to control ?
  - cooling profiles
  - seeding

Classification	Cooling rate (K/h)
Slow	1-5
Realistic	5-10
Fast	10-15
Crash	> 15

Cooling profiles

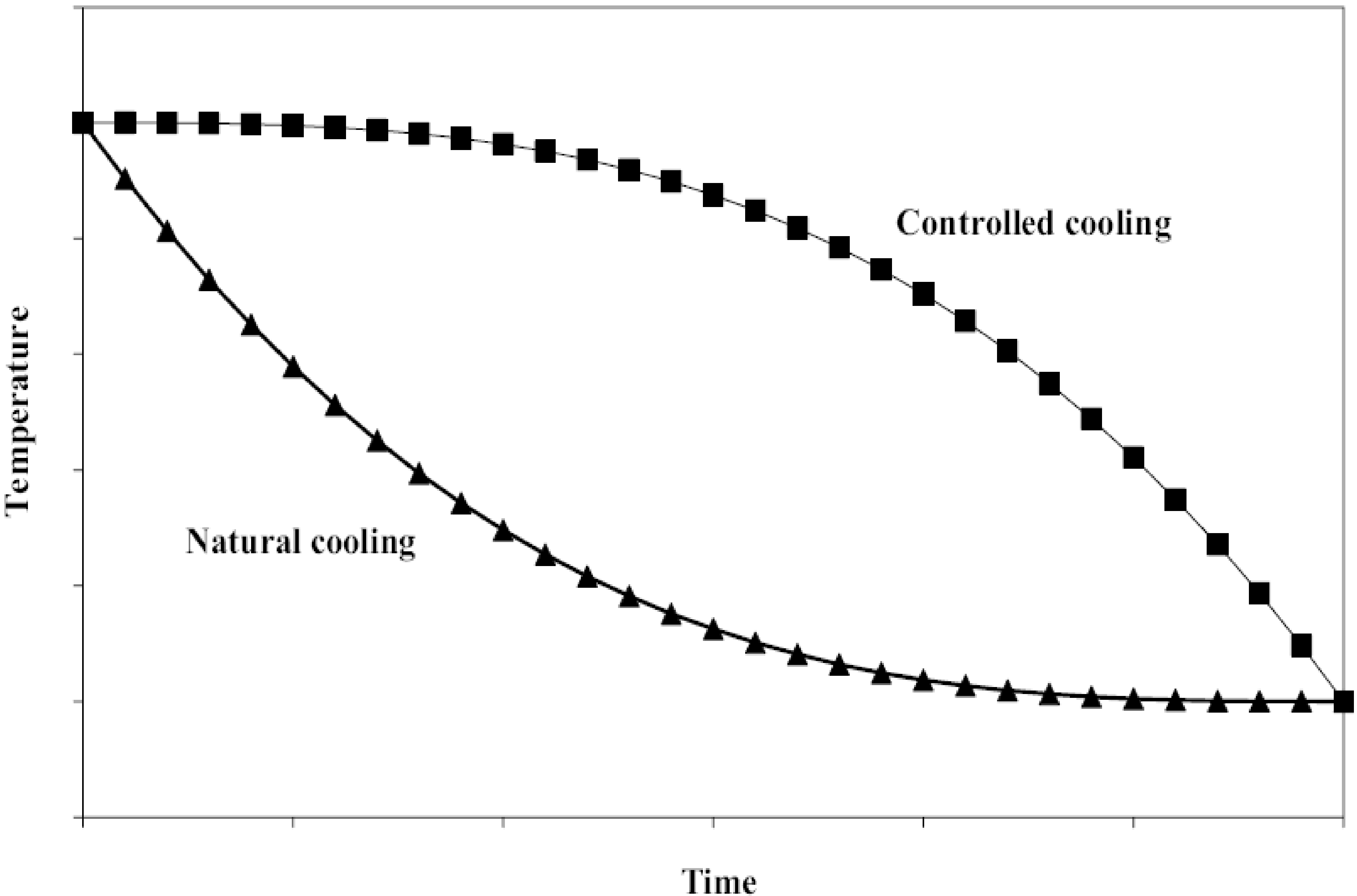
for supersaturated K_2SO_4-solutions in a seeded batch-crystallizer



Omar, W.: *Zur Bestimmung von Kristallisationskinetiken auch unter der Einwirkung von Additiven mittels Ultraschallmeßtechnik*, Dissertation, Universität Bremen, 1999.

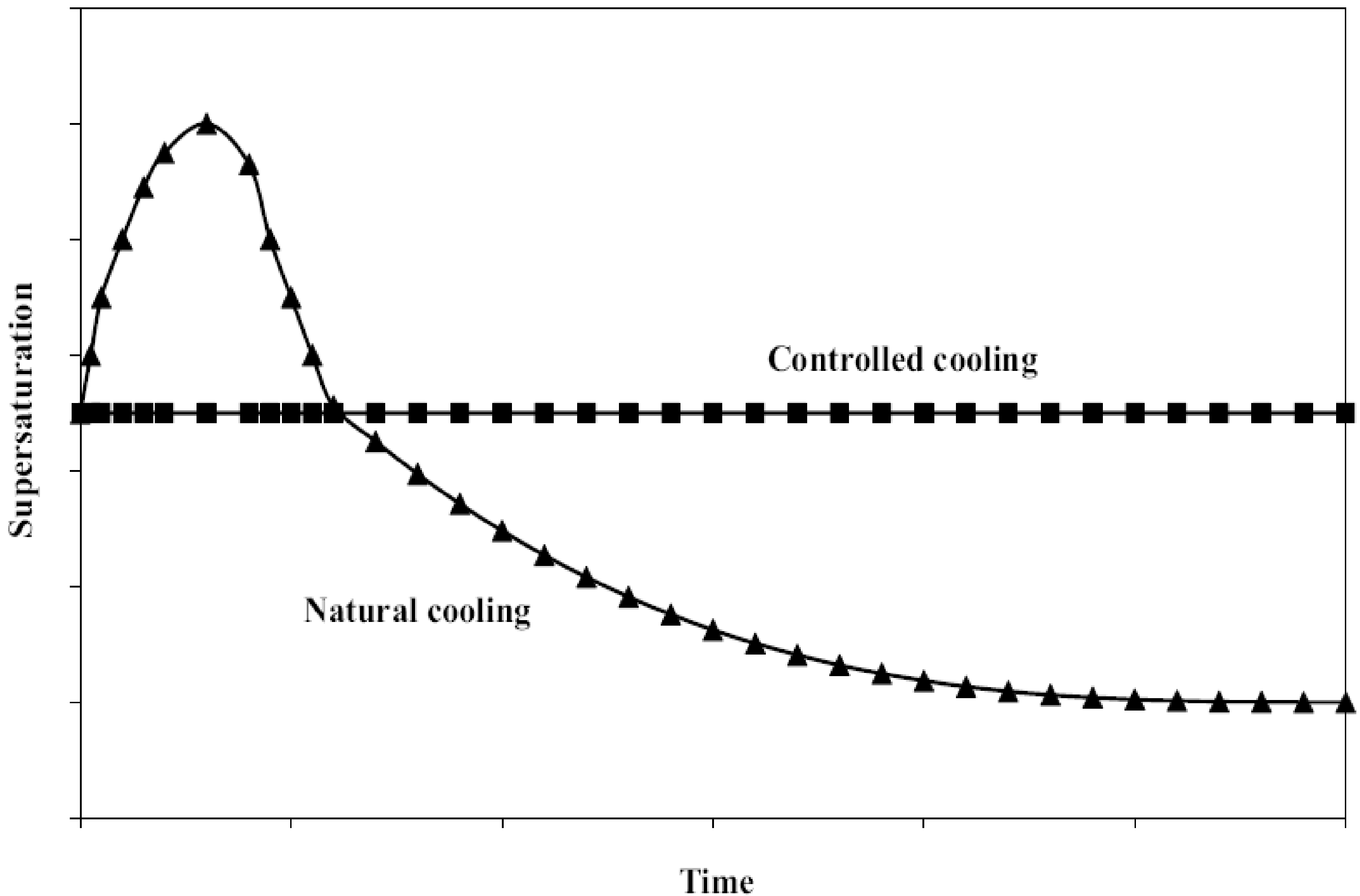
Cooling profiles

temperature profiles at natural and controlled cooling in batch crystallization



Cooling profiles

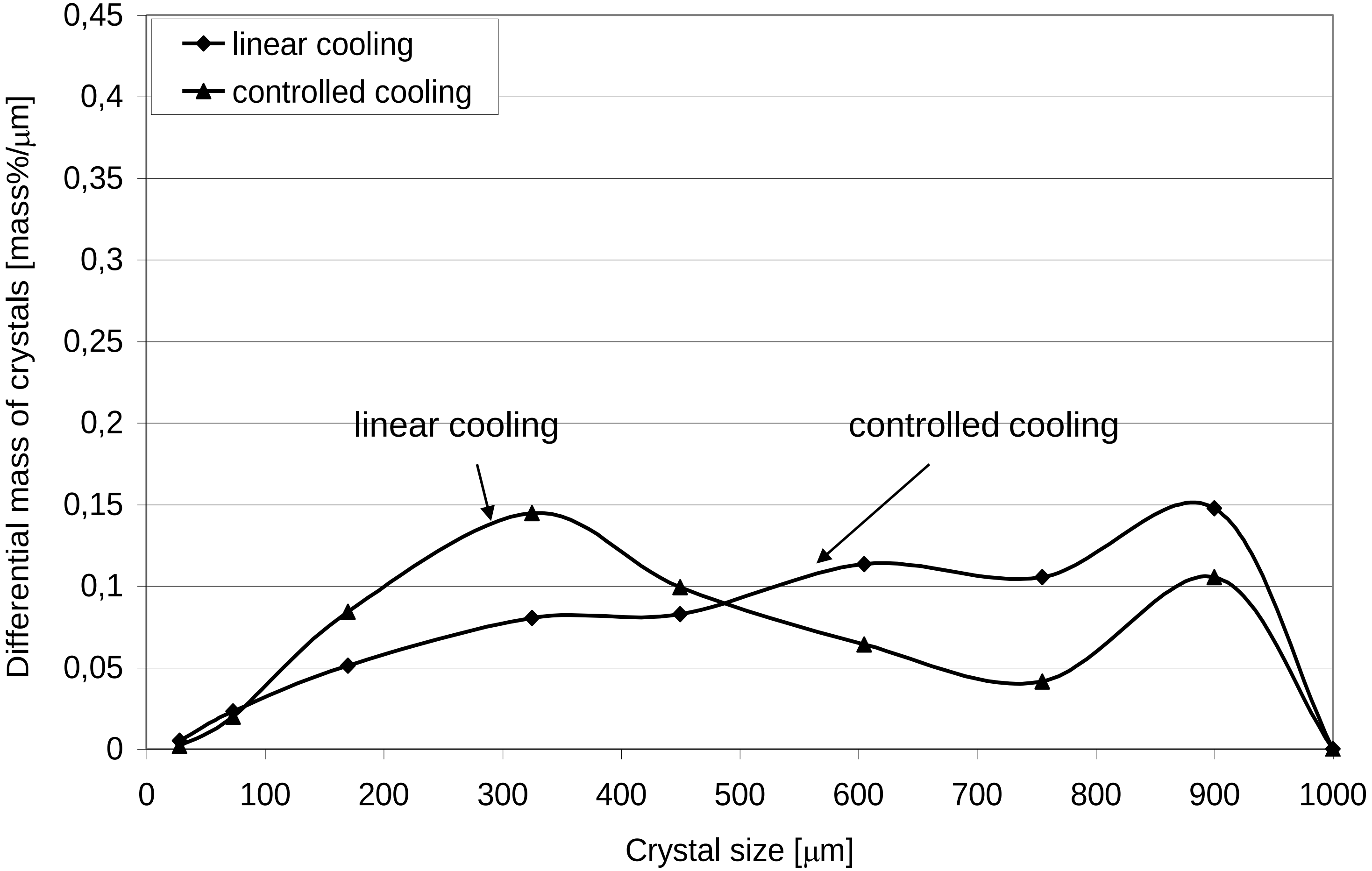
supersaturated profiles at natural and controlled cooling in batch crystallization



Omar, W.: *Zur Bestimmung von Kristallisationskinetiken auch unter der Einwirkung von Additiven mittels Ultraschallmeßtechnik*, Dissertation, Universität Bremen, 1999.

Example

Crystal size distribution obtained for different cooling programs without seeding



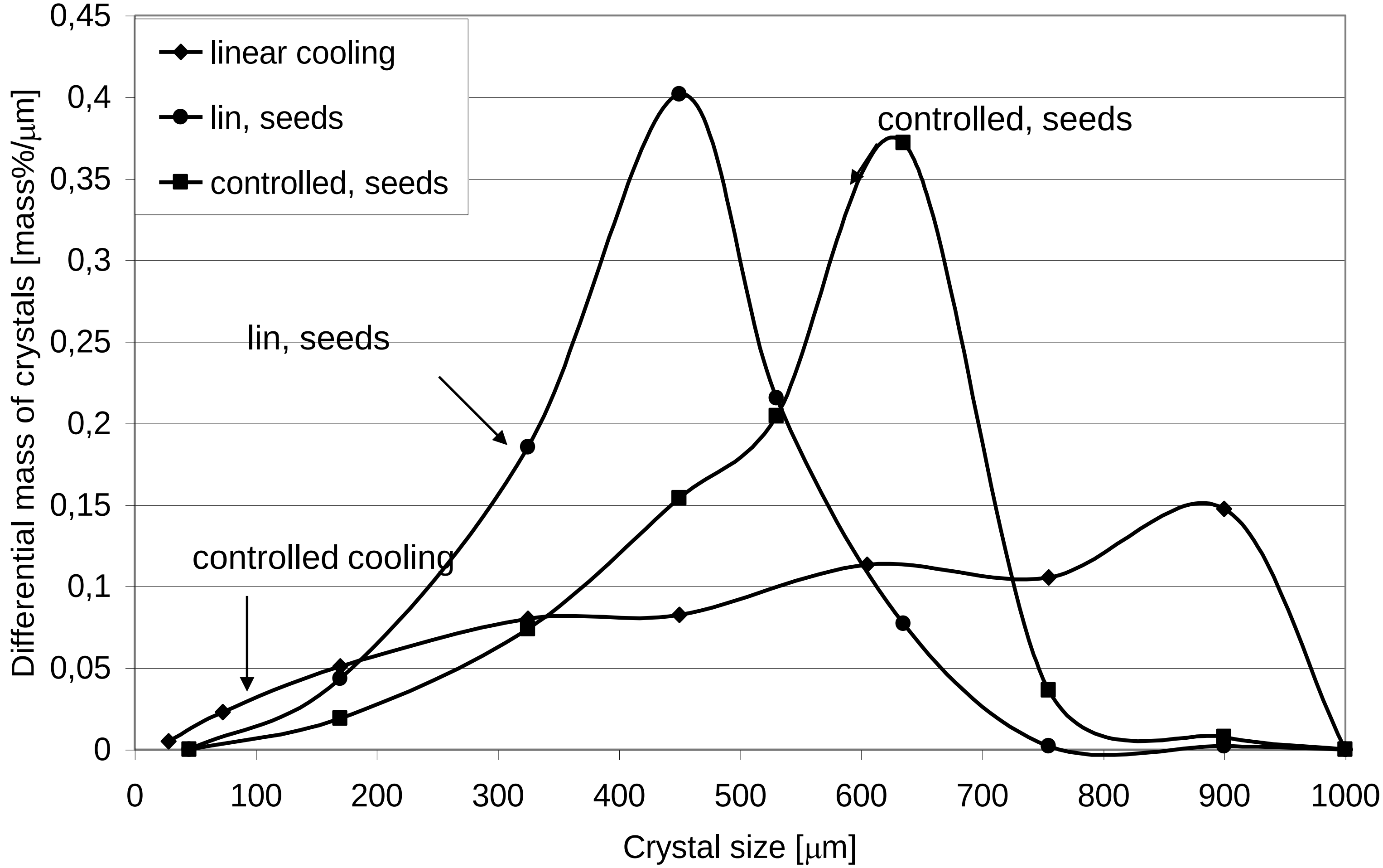
Warstat, A.: *Heuristische Regeln zur Optimierung von Batch-Kühlungskristallisationsprozessen*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/06/07H060/index.htm, 2006.

Example seeding (batch process)

- When – at what supersaturation
- How:
  - seed mass
  - seed size distribution
  - seed quality
- Supersaturation maintaining / changing profile

Example seeding (batch process)

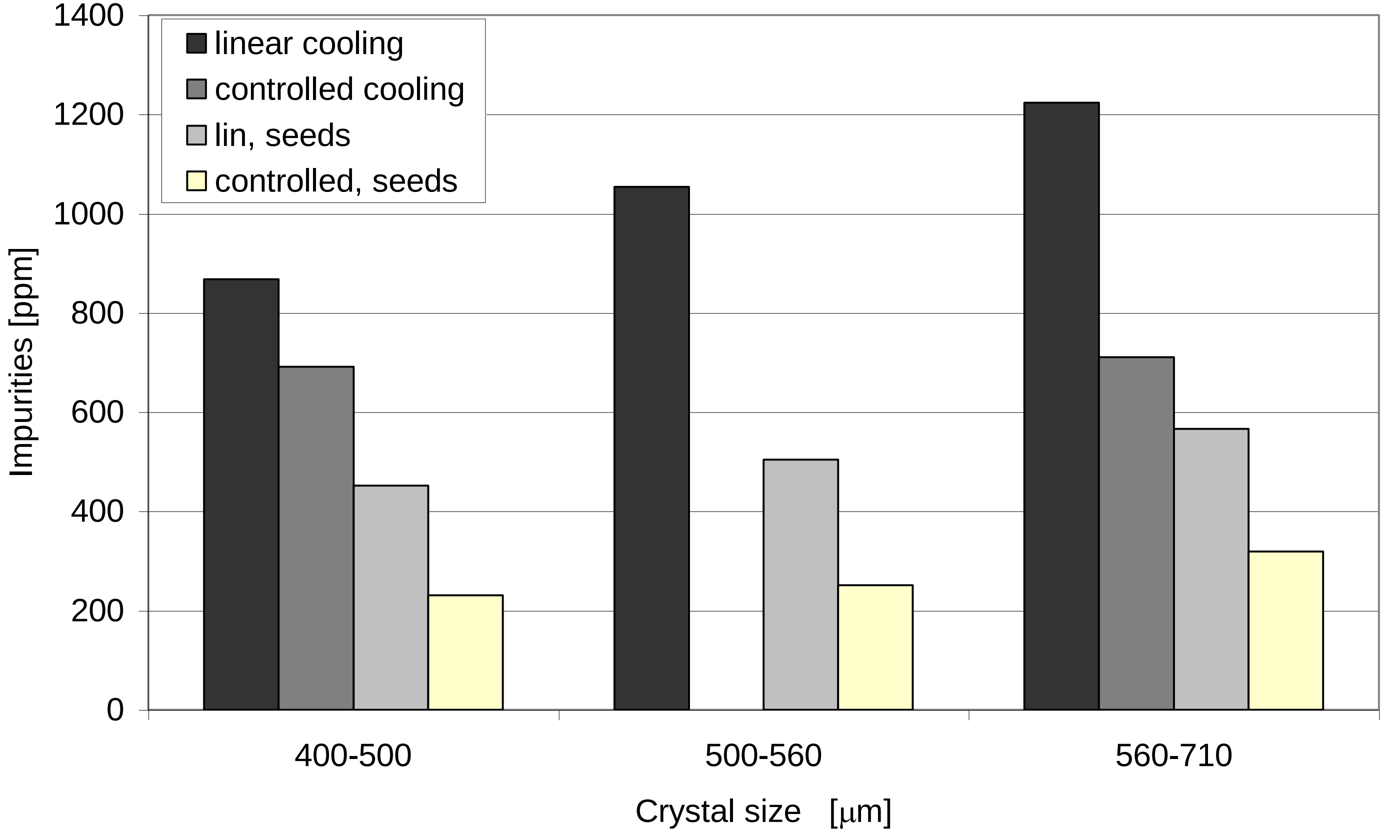
Crystal size distribution: a) different cooling programs, b) with and without seeding



Warstat, A.: *Heuristische Regeln zur Optimierung von Batch-Kühlungskristallisationsprozessen*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/06/07H060/index.htm, 2006.

Example seeding (batch process)

Water content in crystals for different size classes

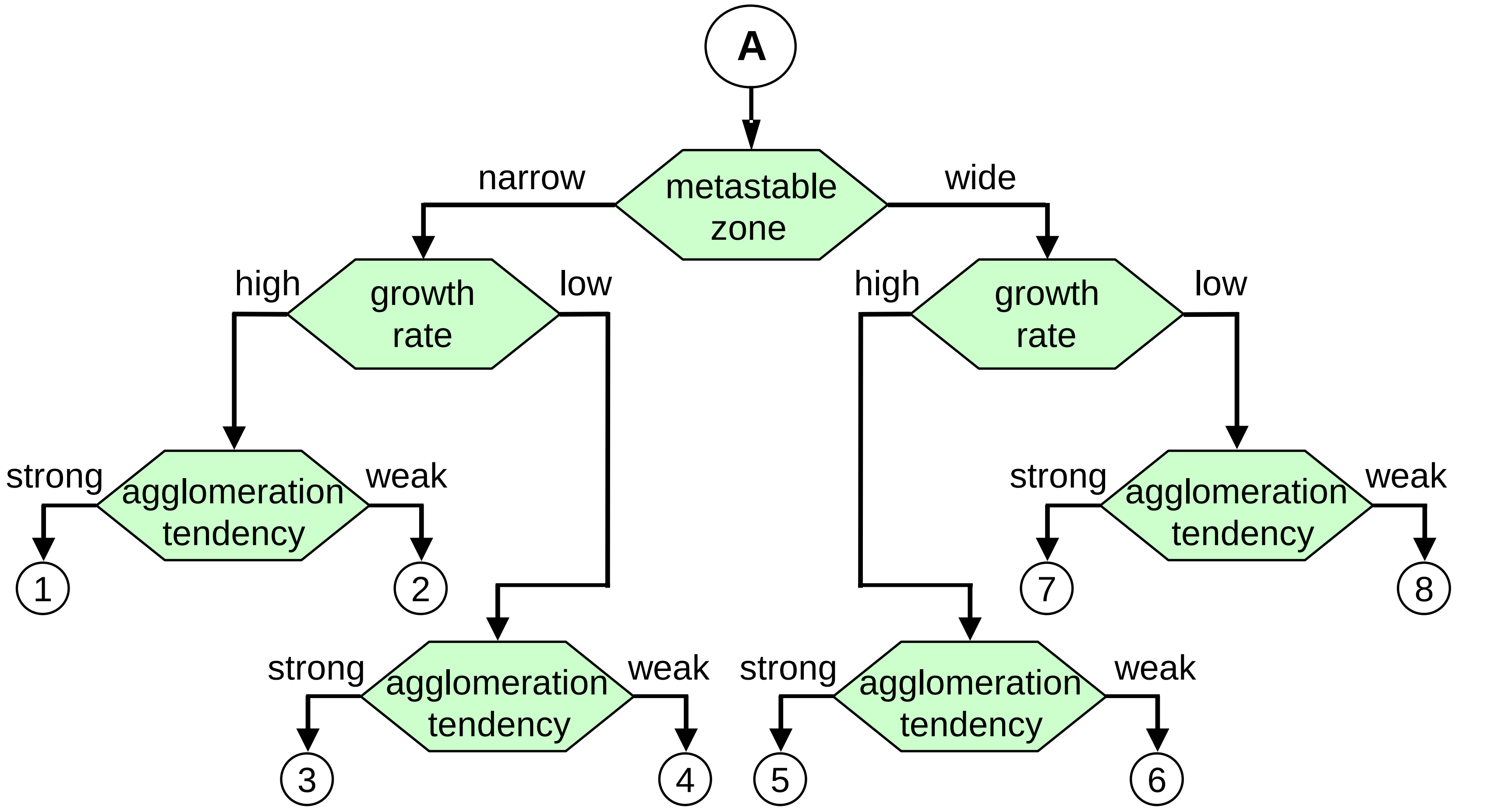


Warstat, A.: *Heuristische Regeln zur Optimierung von Batch-Kühlungskristallisationsprozessen*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/06/07H060/index.htm, 2006.

Example

seeding (batch process)

- Decision tree; target value „unimodal crystal size distribution and purity“ [2]

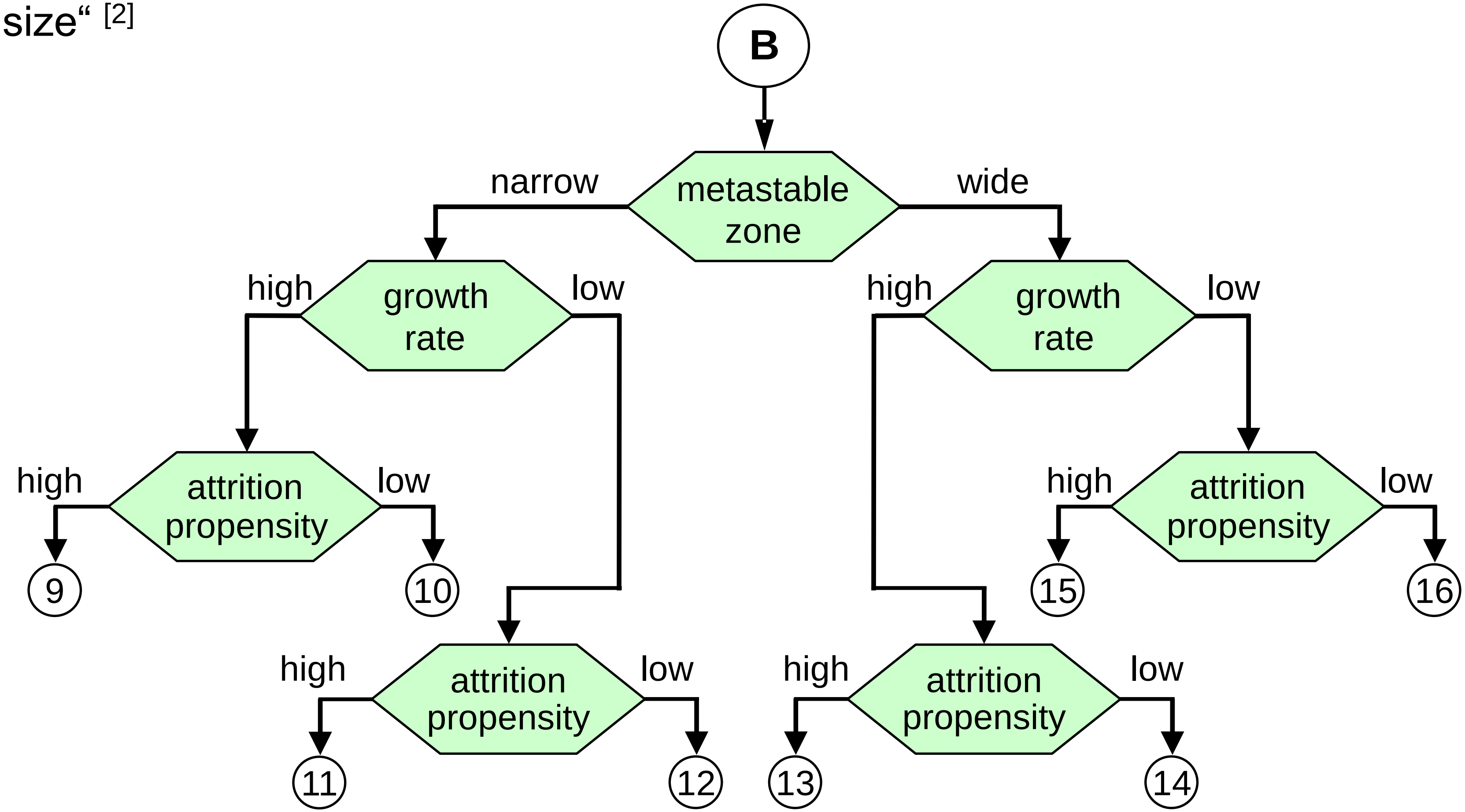


[2] Warstat, A.: *Heuristische Regeln zur Optimierung von Batch-Kühlungskristallisationsprozessen*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/06/07H060/index.htm, 2006.

Example

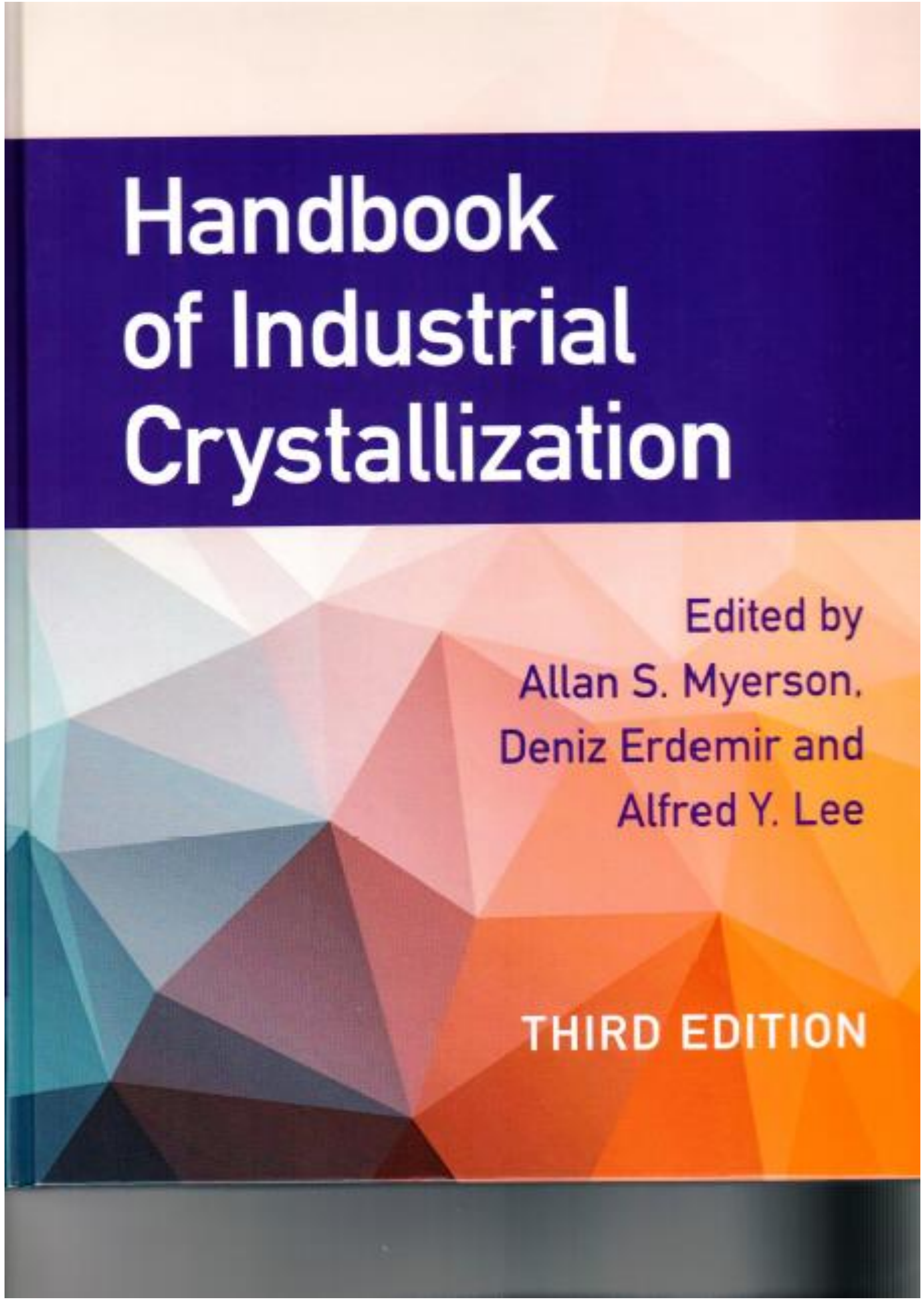
seeding (batch process)

- Decision tree; target value „unimodal crystal size distribution and specific crystal size“ [2]



[2] Warstat, A.: *Heuristische Regeln zur Optimierung von Batch-Kühlungskristallisationsprozessen*, PhD-thesis, Martin-Luther-Universität Halle-Wittenberg, online-publication: http://sundoc.bibliothek.uni-halle.de/diss-online/06/07H060/index.htm, 2006.

Thank you for your attention!


Handbook
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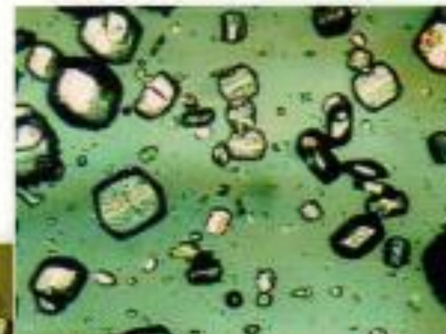
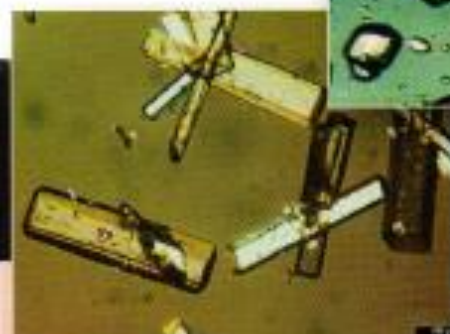
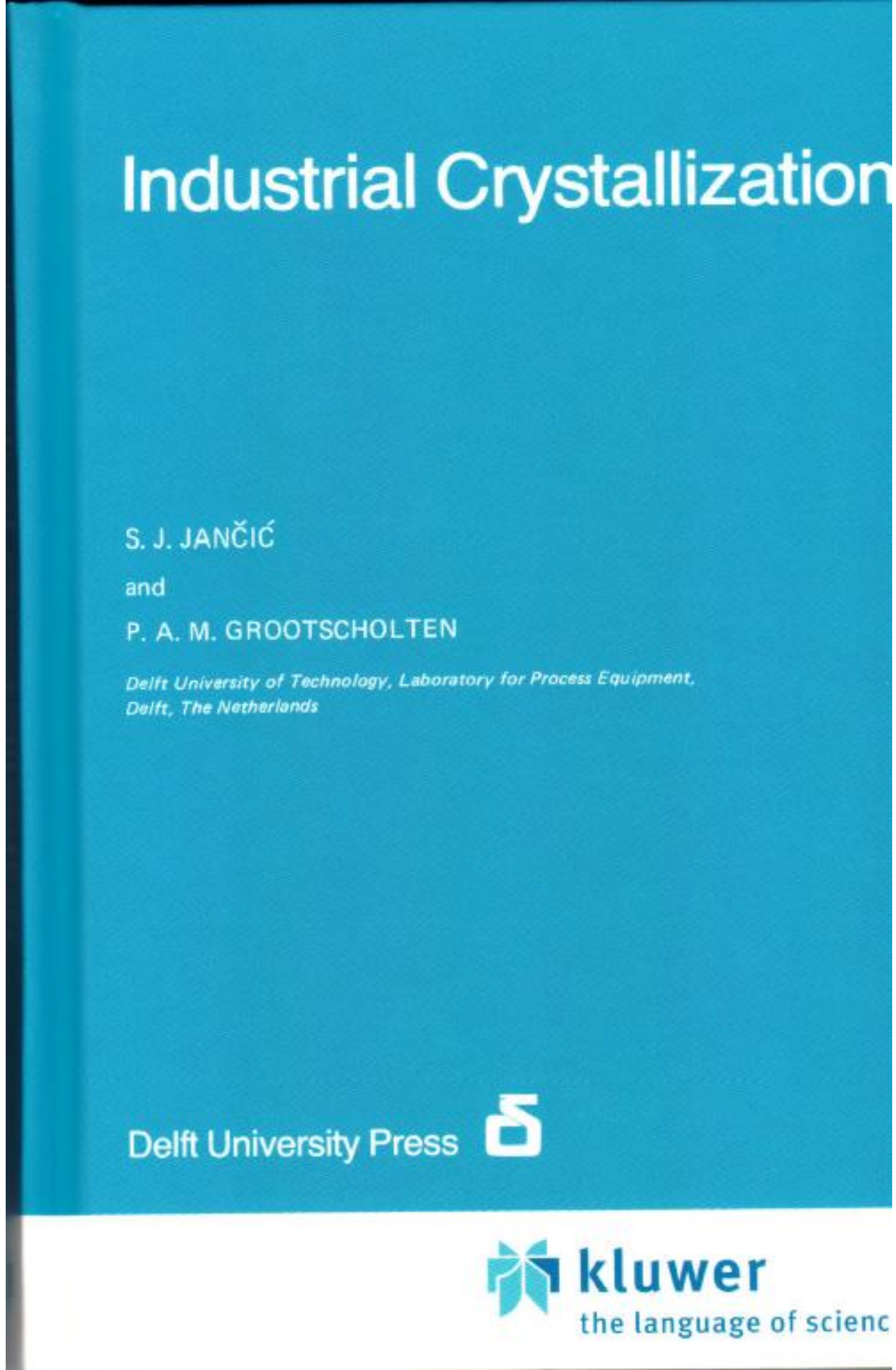
Edited by
Allan S. Myerson,
Deniz Erdemir and
Alfred Y. Lee
THIRD EDITION

Crystallization

Basic Concepts and Industrial Applications



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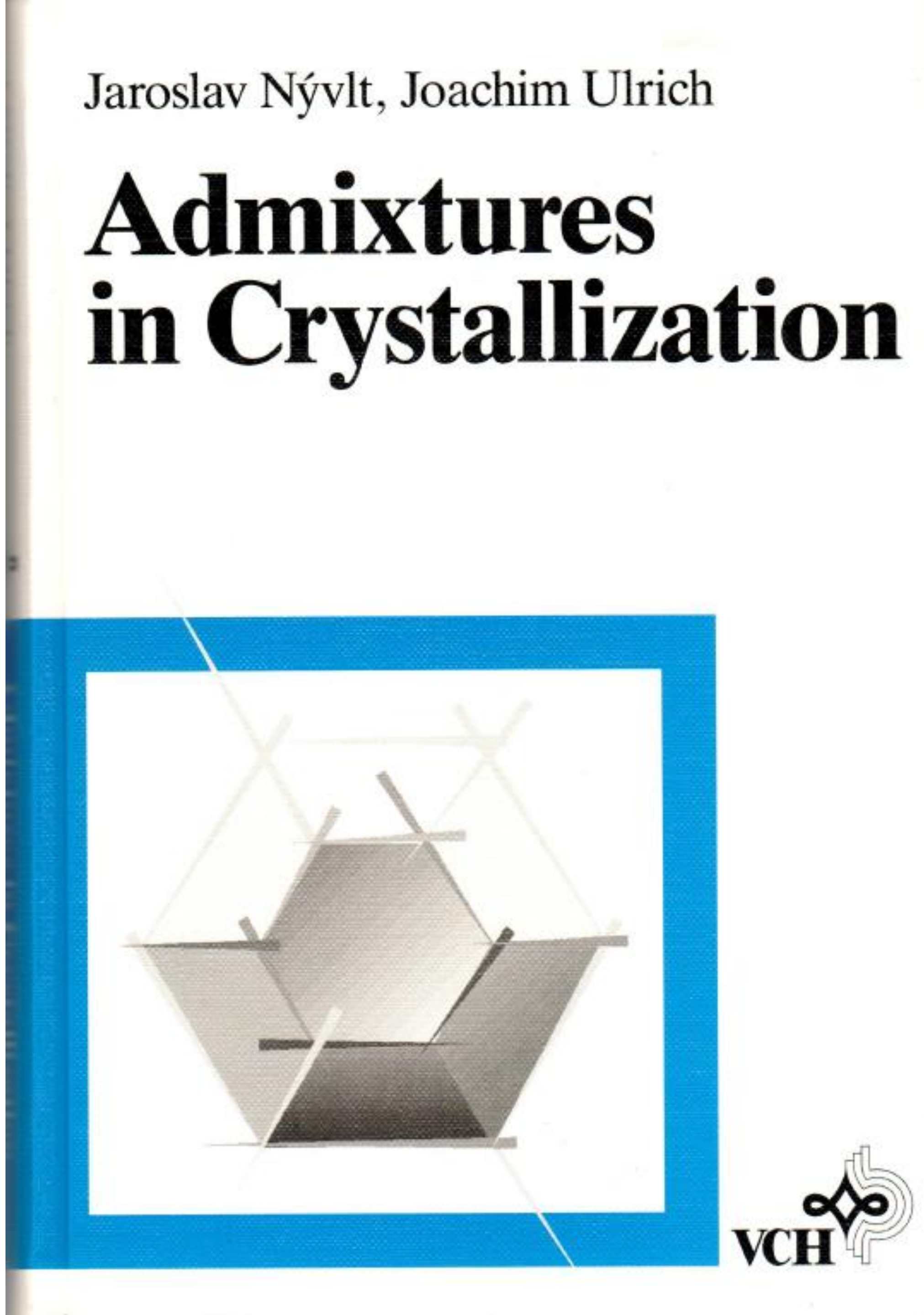
and
P. A. M. GROOTSCHOLTEN
Delft University of Technology, Laboratory for Process Equipment,
Delft, The Netherlands
Delft University Press
kluwer
the language of scienc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Admixtures in Crystallization
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CRYSTALLIZATION

Second Edition

TECHNOLOGY

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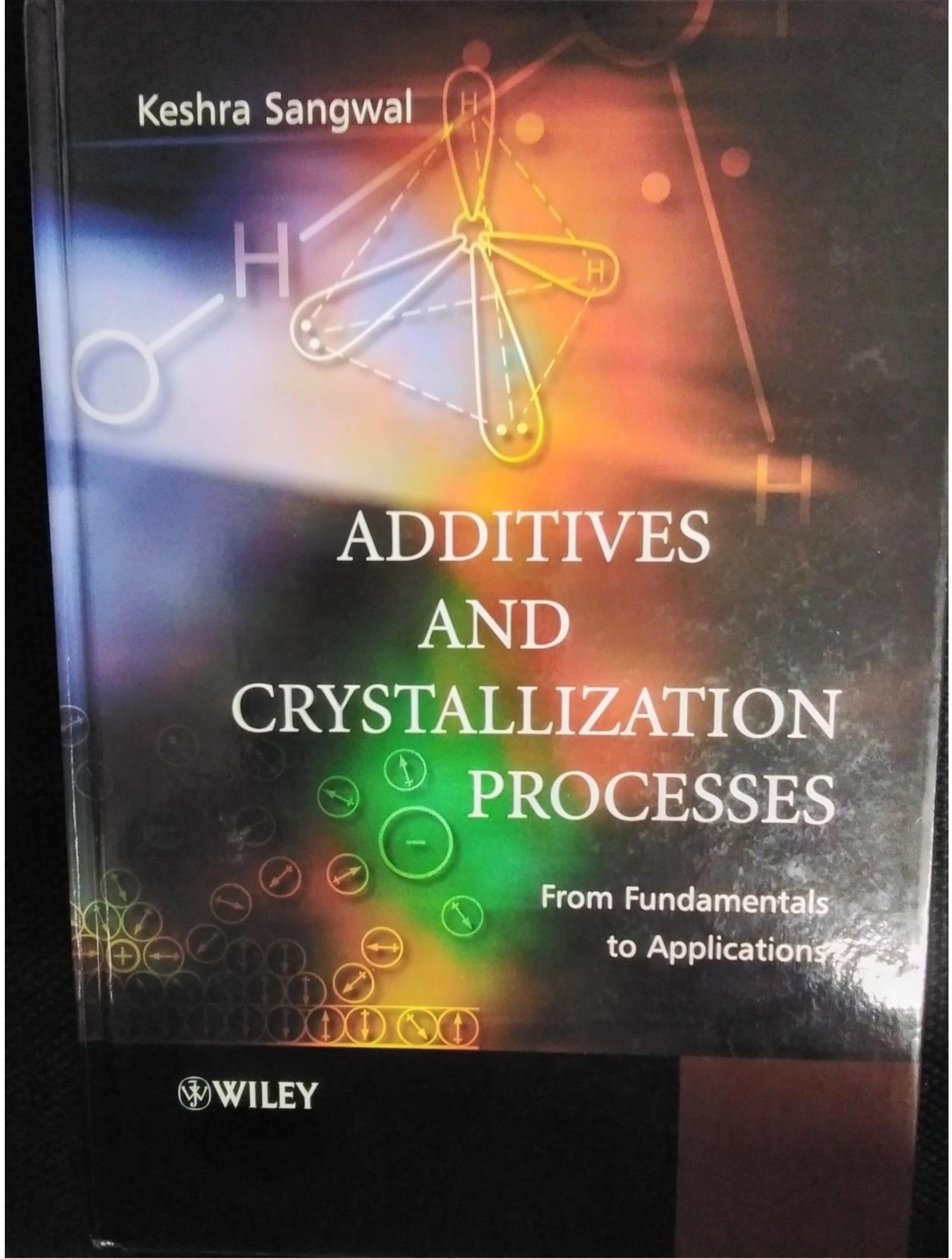
Keshra Sangwal
ADDITIVES
AND
CRYSTALLIZATION
PROCESSES
From Fundamentals
to Applications
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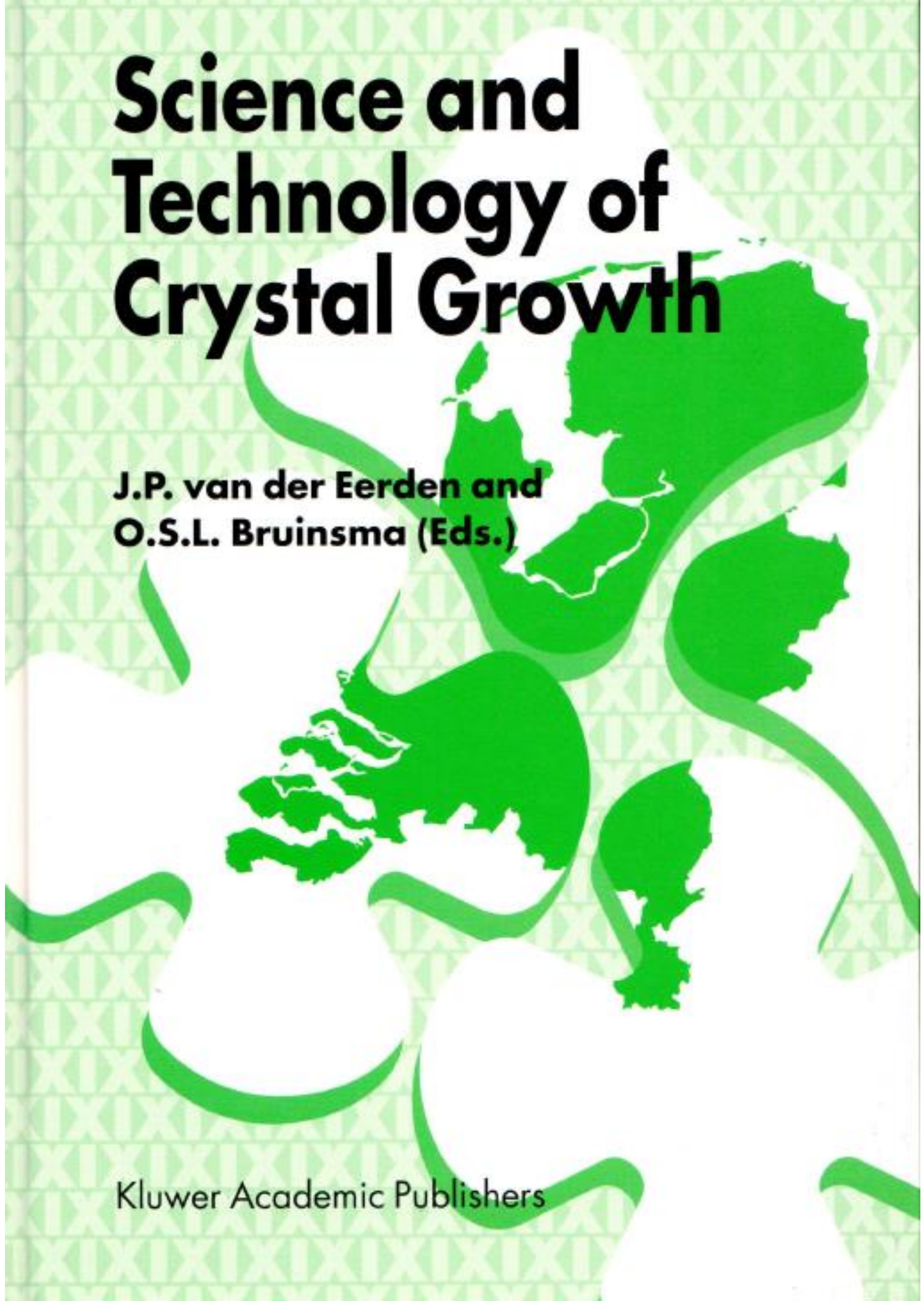

Science and
Technology of
Crystal Growth
J.P. van der Eerden and
O.S.L. Bruinsma (Eds.)
Kluwer Academic Publishers

Thank you for your attention!

Martin Luther University Halle-Wittenberg
Center of Engineering Science
Department of Thermal Separation Processes

Protein Crystals

-more questions than answeres-

Prof. Dr. Dr. h.c. Joachim Ulrich

Crystals

"Crystals are solids in which the atoms are arranged in a periodic repeating pattern that extends in three dimensions.

While all crystals are solids, not all solids are crystals.

Materials that have short-range rather than long-range ordering, like glass, are non-crystalline solids.

A non-crystalline solid is often referred to as an amorphous solid" [MYE02].

Myerson, A.S., Ginde, R., Crystals, Crystal Growth, and Nucleation, In: Handbook of Industrial Crystallization, edited by Allan S. Myerson, Butterworth-Heinemann, USA, 2002, 33-65, ISBN: 0-7506-7012-6.

Polymorphism, Hydrates, Solvates

Grant **[GRA99, Loh06]** defines *polymorphism, amorphous solids and solvates* as:

"**Polymorphism** is often characterized as the ability of one drug substance (chemical compound) to exist in two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice".

*"**Solvates*** are crystalline solid adducts containing either stoichiometric or nonstoichiometric amounts of a solvent incorporated within the crystal structure. If the incorporated solvent is water, the solvates are also commonly known as ***hydrates***".

Grant, D.J.W., Theory and Origin of Polymorphism, In: Polymorphism in Pharmaceutical Solids, edited by Harry G. Brittain, Marcel Dekker Inc., New York, 1999, 1-34, ISBN: 0-8247-0237-9.
Lohani, S., Grant, D.J.W., Thermodynamics of Polymorphs, In: Polymorphism in the Pharmaceutical Industry, edited by Rolf Hilfiker, Wiley-VCH, Weinheim, 2006, 21-41, ISBN: 978-3-527-31146-0.

Phase diagrams

“A phase diagram graphically represents (in two or three dimensions) the equilibria between various phases of a system in a wide range of temperature, pressure and concentration/composition.

It specifies the equilibrium conditions (T, p and x) and the corresponding phase present at this state.

Thus, in case of SLE, the phase diagram also tells about the solid phases occurring in a system, such as polymorphs, solvates or intermediate compounds” [LOR13].

Lorenz, H., Solubility and Solution Equilibria in Crystallization, In: Crystallization – Basic Concepts and Industrial Application, edited by Wolfgang Beckmann, Wiley-VCH, Weinheim, 2013, 35-74, ISBN: 978-3-527-32762-1.

Introduction

protein crystallization

special characteristics of protein crystallization[1), 2)]

multi-component system

complexity / diversity

often restricted crystallization conditions

intermolecular interactions

(limited solubility data, risk of denaturation, loss of activity)

(e.g. hydrogen bonds, salt bridges, hydrophobic forces)

very high supersaturation required

crystallization / solubility

(temperature, buffer pH, solvent composition, crystallizing agent e.g. salt, ionic strength)

slow nucleation

(macromolecules, asymmetry)

crystal lattice

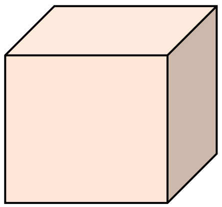
(large number of contacts between the molecules, crystal channels)

[1)] McPherson A.: Crystallization of biological macromolecules; *Cold Spring Harbor Laboratory Press, Cold Spring* 1998.

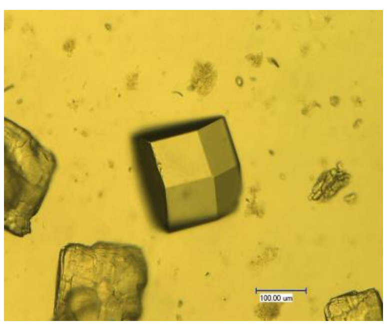
[2)] Banga A.K.:Therapeutic peptides and proteins; second edition, *CRC Press* 2006.

production conditions of different lysozyme crystal morphologies

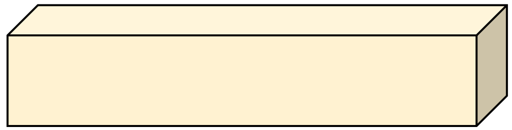
Tetragonal[5)]



acetate buffer pH5,
50 mg/mL,
4 wt% NaCl, **4 ˚C**

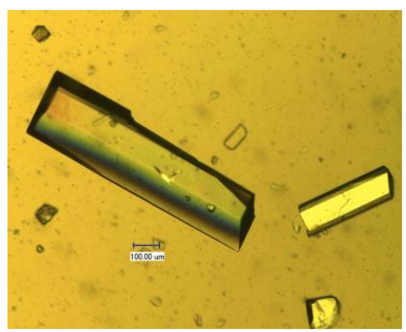


HTO[5)]

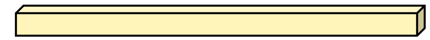


High Temperature Orthorhombic

acetate buffer pH5,
115 mg/mL,
6 wt% NaCl, **37 ˚C**

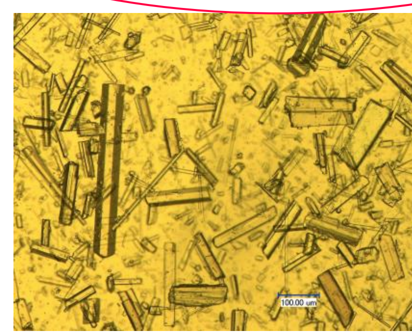


LTO[5)]



Low Temperature Orthorhombic

glycine buffer pH9.6,
50 mg/mL,
4 wt% NaCl, **20 ˚C**



→ **different crystallization conditions → different lysozyme crystal morphologies**

[5)] AlDabaihbeh, N. *PhD Thesis*, Department of Chemical & Biological Engineering, Illinois Institute of Technology, 2010.

analysis of lysozyme crystals

4 main components

1) **lysozyme** (protein)
   - **protein concentration**
   - **activity**
   - XRPD (powder x-ray diffraction)

2) **buffer solution** (high solvent content)
   - **dyeing / coloring**
   - pH

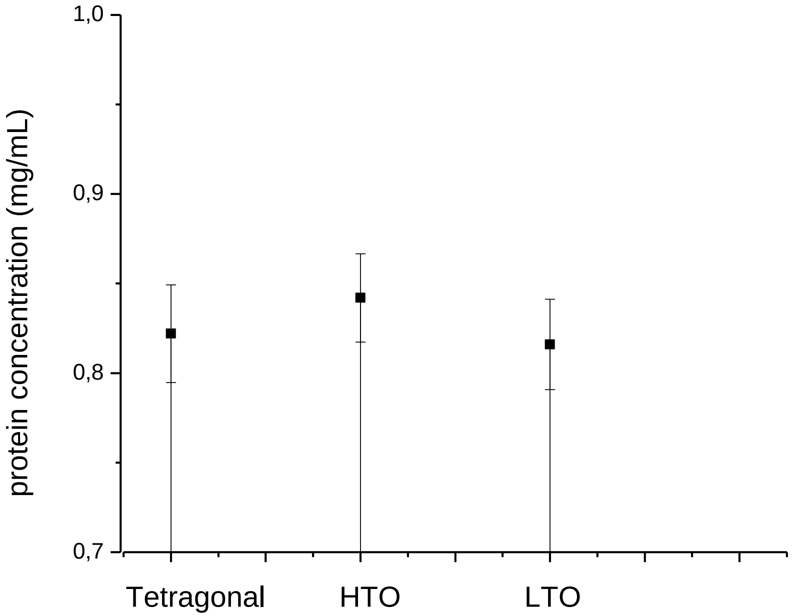
3) **free + bound water** (solvent content)
   - **TGA** (thermo gravimetric analysis)

4) **NaCl** (crystallizing agent)
   - conductivity
   - silver nitrate (precipitation)
   - **EDX** (energy dispersive x-ray spectroscopy)
   - XRPD

1) lysozyme (protein)

protein concentration + enzymatic activity

protein concentration (mg/mL)



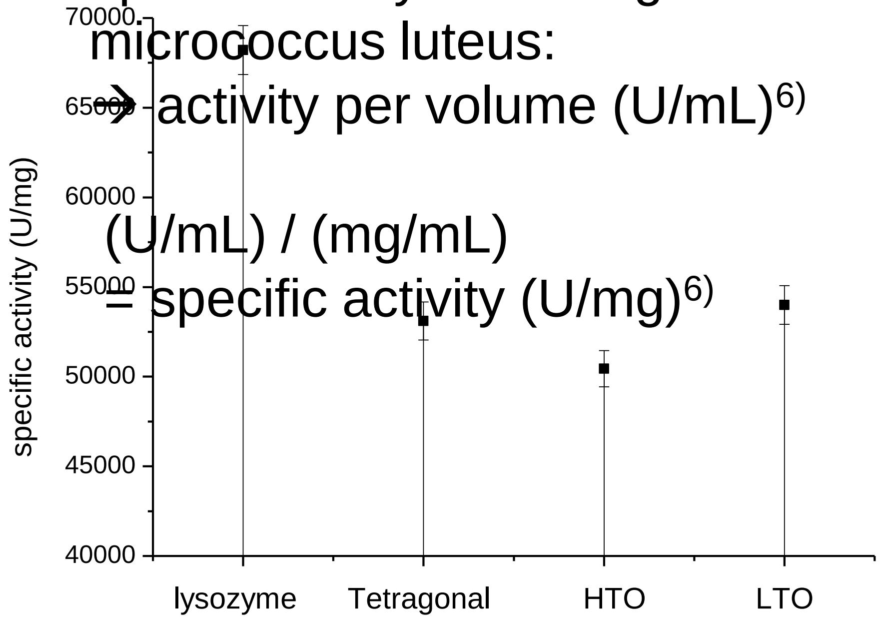
specific enzymatic activity (U/mg)[7)]

optical activity test using micrococcus luteus:

→ activity per volume (U/mL)[6)]

(U/mL) / (mg/mL)

= specific activity (U/mg)[6)]



→ **highest protein content: HTO > Tetragonal > LTO**

→ **specific activity: LTO > Tetragonal > HTO**

6) Löffler G.: *Basiswissen Biochemie*, 5. Auflage, Springer 2003

7) Shugar D.: *Biochimica et Biophysica Acta 8* (1952) 302-309

2) buffer solution (free water)

dyeing / coloring

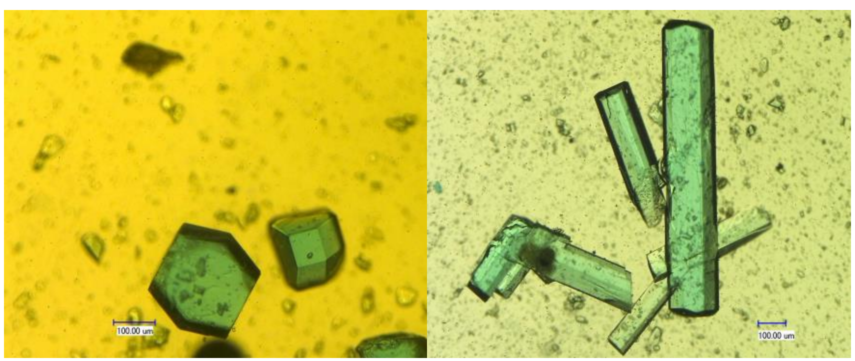
Tetragonal, HTO and LTO lysozyme crystals

+ methylene blue

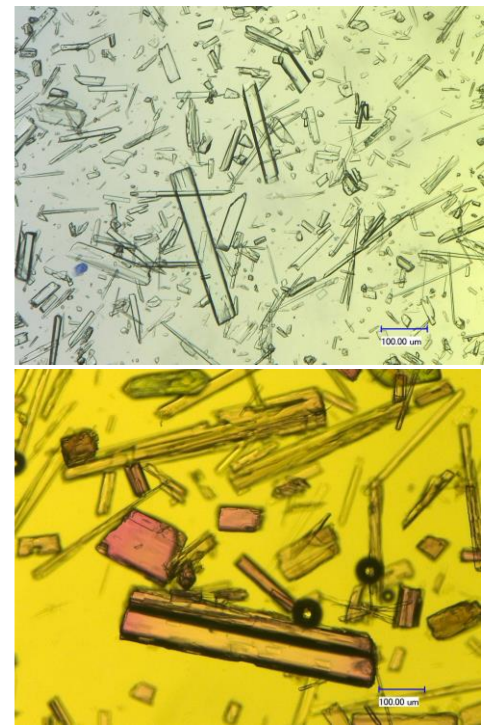
Tetragonal

after 24h

HTO



LTO



→ **buffer solution inside the crystals**

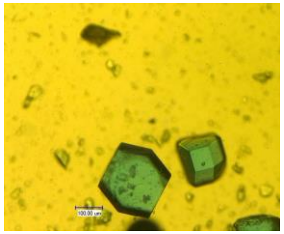
+ phenolphthalein after 24h

Müller, C., How to describe protein crystals correctly? –case study of lysozyme crystals-, Dissertation, Martin-Luther-Universität Halle-Wittenberg, 2012, http://141.48.65.178/hs/content/titleinfo/1177647.

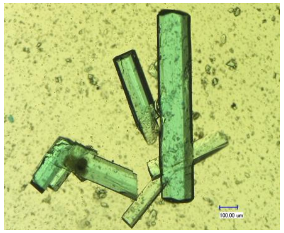
2) buffer solution (free water)

dyeing / coloring

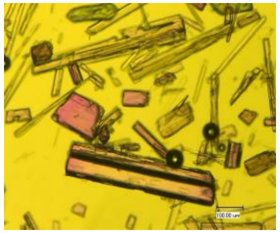
Tetragonal



HTO



LTO

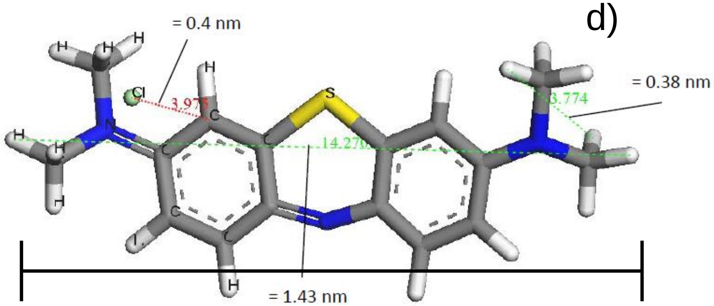


why?

→ size and shape of the dye molecule and crystal pores

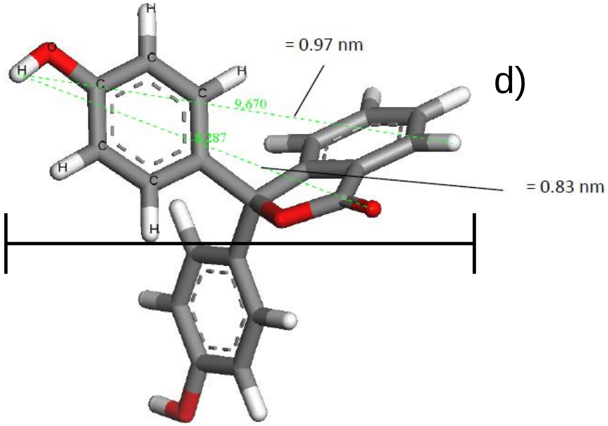
methylene blue

1.43 nm



phenolphthalein

0.97 nm

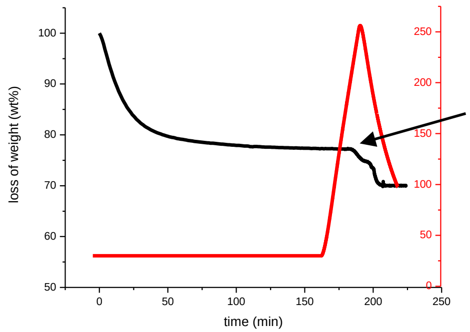


d) Accelyrs Software Inc., Material Studio 4.0, San Diego USA 2005

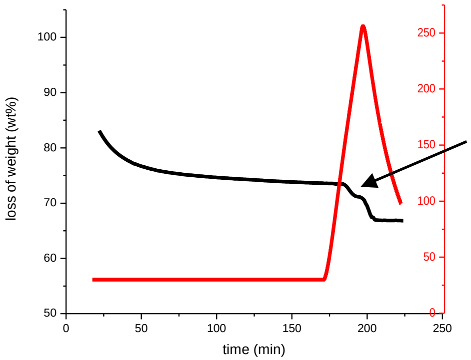
3) free (buffer)+ bound water

TGA[e)]

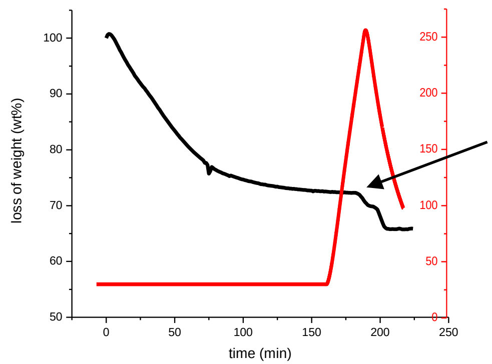
Tetragonal



HTO



LTO



1. step: free water molecules (buffer in the void spaces)

2. step: bonded water molecules (crystal lattice)

decomposition

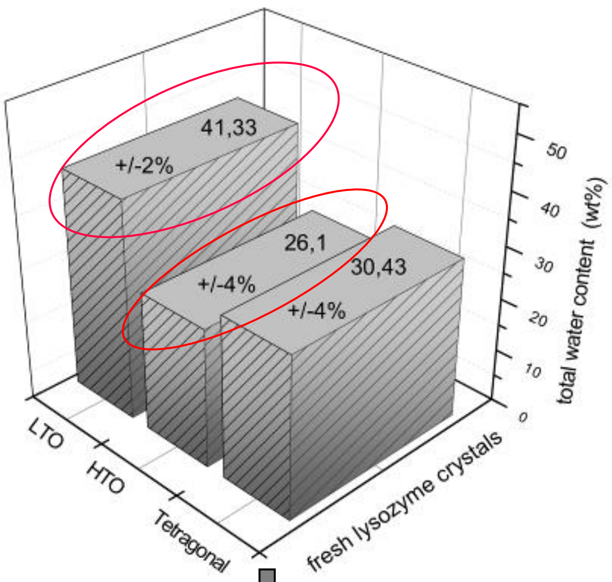
→ two “types“ of water (free and bound)

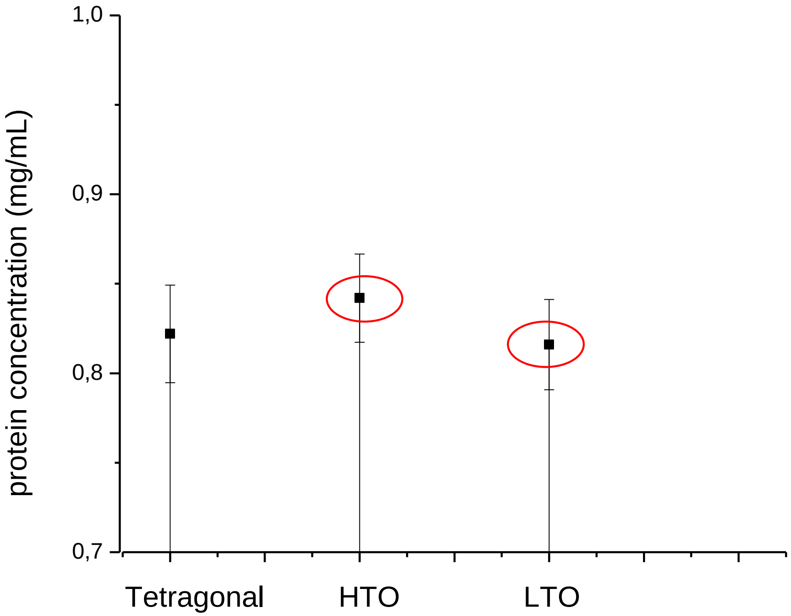
e) TGA _ Thermogravimetric analysis

8) Petrova et al.: *Crystallography Reports*, 52, 2 (2007), 275-279

3) free (buffer) + bound water
TGA[e)] results

1. + 2. step: loss of water
→ total water content of fresh lysozyme crystals by TGA





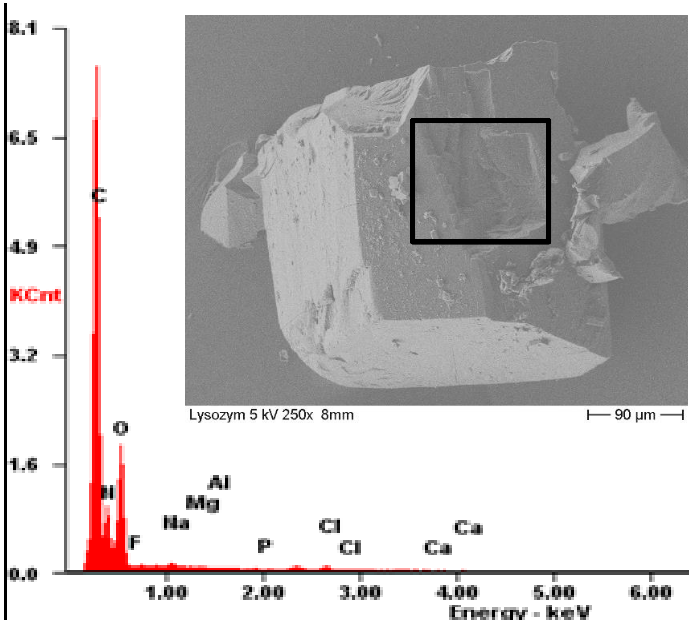
→ total water content varies
→ LTO > Tetragonal > HTO

Müller, C., How to describe protein crystals correctly? –case study of lysozyme crystals-, Dissertation, Martin-Luther-Universität Halle-Wittenberg, 2012, http://141.48.65.178/hs/content/titleinfo/1177647.

4) NaCl (crystallizing agent)

EDX[f)]

➢ Tetragonal:



surface sample

Element	wt%	at wt%
C	63.90	70.16
N	15.50	14.59
O	16.40	13.51
F	00.07	00.05
Na	**00.39**	**00.22**
Mg	00.00	00.00
Al	00.11	00.05
P	00.00	00.00
S	01.53	00.63
Cl	**02.00**	**00.74**
Ca	00.11	00.04

interior sample

Element	wt%	at wt%
C	63.53	70.54
N	14.88	14.17
O	15.12	12.60
F	00.09	00.06
Na	**00.58**	**00.34**
Mg	00.03	00.02
Al	00.03	00.01
P	00.02	00.01
S	02.38	00.99
Cl	**03.28**	**01.23**
Ca	00.06	00.02

→ Tetragonal lysozyme crystals contain NaCl

→ no homogenity

→ same is assumed for HTO and LTO

f)EDX _ Energy dispersive X-ray Spectroscopy by REM Jeol SM 7401 F with the EDX System genesis of EDAX

4) crystallizing agent

In the paper:

„Growth Inhibition of Protein Crystals: A study of Lysozyme Polymorphs“

of Heijna, M.C.R., van Enckevort, W.J.P., Vlieg., E. [Hej08],

the following sentence is to be found:

„Note:

The various possible crystal structures of lysozyme are not polymorphs in the strictest sense of the word, because the salts are incorporated in the crystal and thus induce different compositions.“

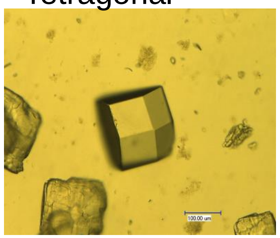
Heijna, M.C.R., van Enckevort, W.J.P., Vlieg., E., Growth Inhibition of Protein Crystals: A Study of Lysozyme Polymorphs, Crystal Growth & Design, 1 (2008) 8, 270-274.

analysis of lysozyme crystals - conclusion

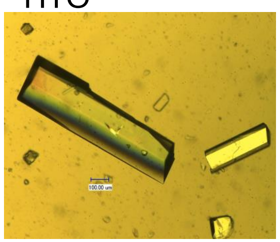
4 main components

lysozyme crystals → **4 components**

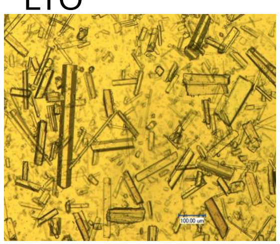
Tetragonal



HTO



LTO



no polymorphism

→ ≠ crystallization conditions – Tetragonal, HTO and LTO morphologies show **variations in the component fractions**

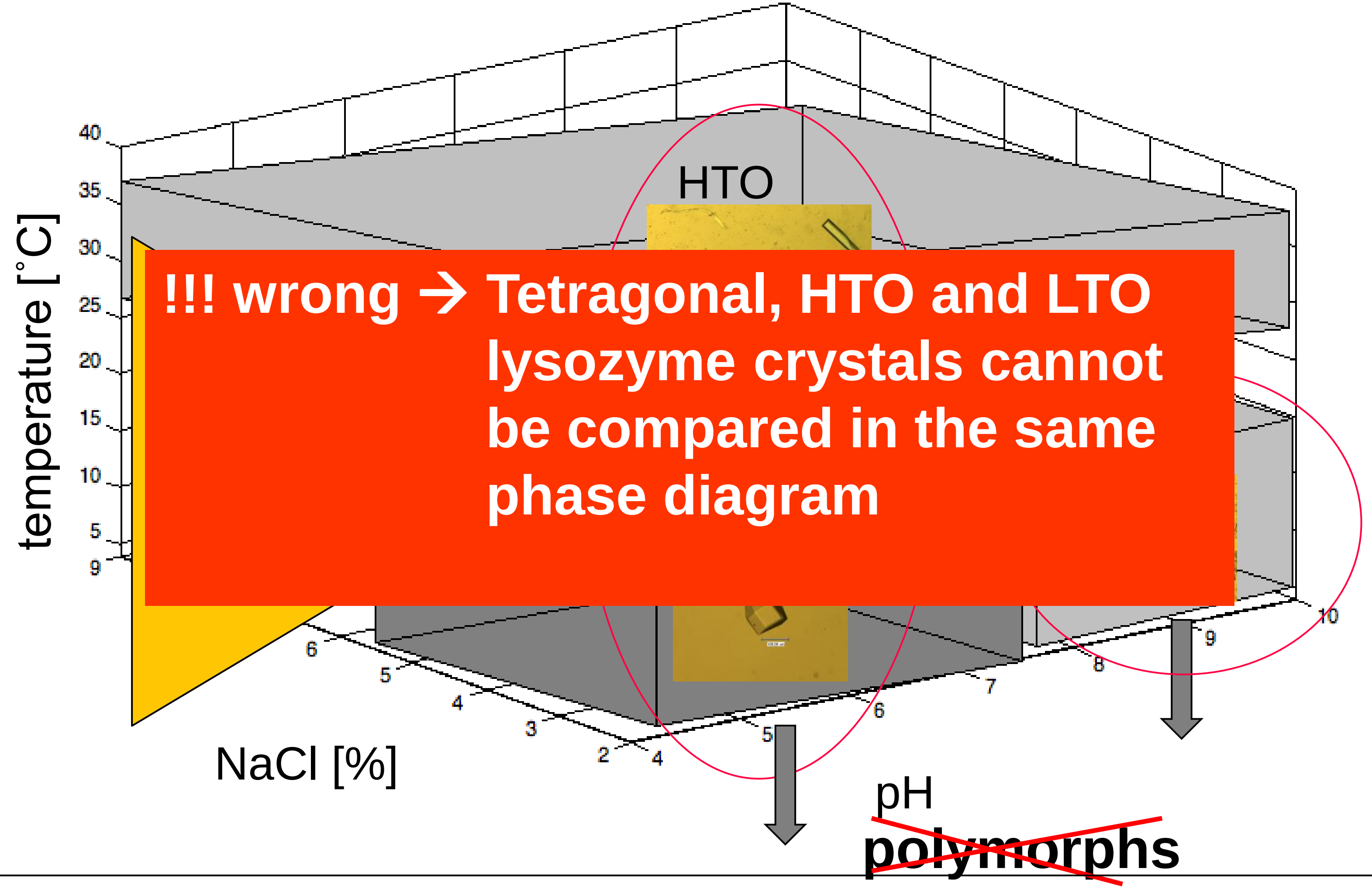
phase diagram !

Tetragonal - HTO → “pseudo“- solvates

LTO → chemically a different solvate

solubility of lysozyme crystals

phase diagram[5)]



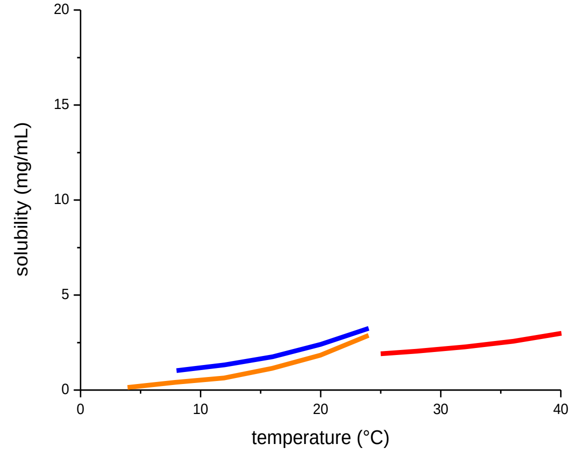
[5)]N. AlDabaihbeh: PhD Thesis, Department of Chemical & Biological Engineering, Illinois Institute of Technology, 2010
Müller, C., How to describe protein crystals correctly? –case study of lysozyme crystals-, Dissertation, Martin-Luther-Universität Halle-Wittenberg, 2012,, http://141.48.65.178/hs/content/titleinfo/1177647

solubility of lysozyme crystals

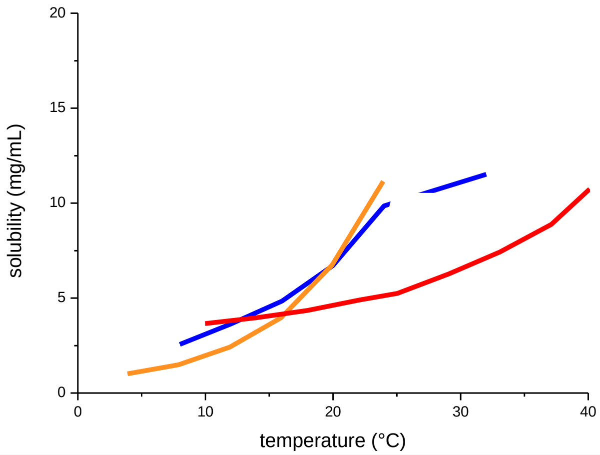
→ new LTO solubility data

--- Tetragonal
--- HTO
--- LTO

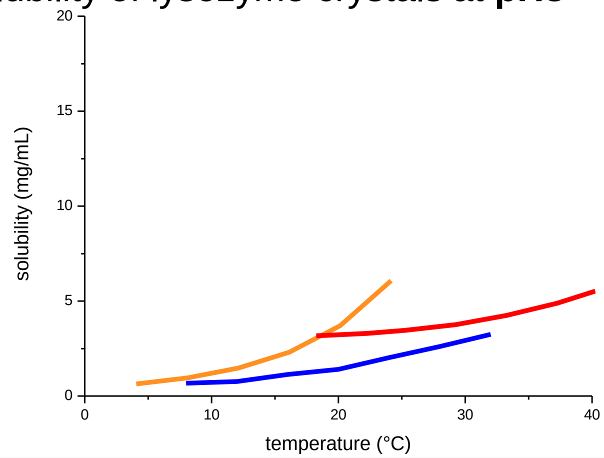
solubility of lysozyme crystals at **pH5.7 + 7 wt%**



solubility of lysozyme crystals at **pH5.7 + 3 wt%**



solubility of lysozyme crystals at **pH8 + 3 wt%**

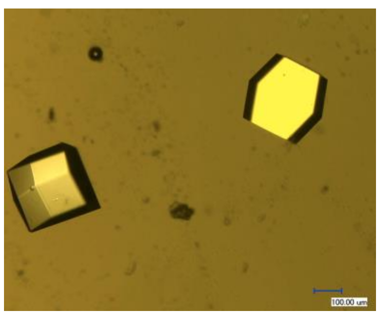


production conditions of different lysozyme crystal morphologies

Tetragonal

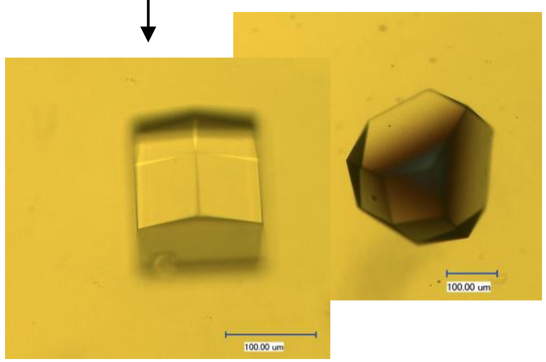
acetate buffer pH5,
50 mg/mL,
4 wt% **salt**, 4˚C

KCl, 24h



Tetragonal

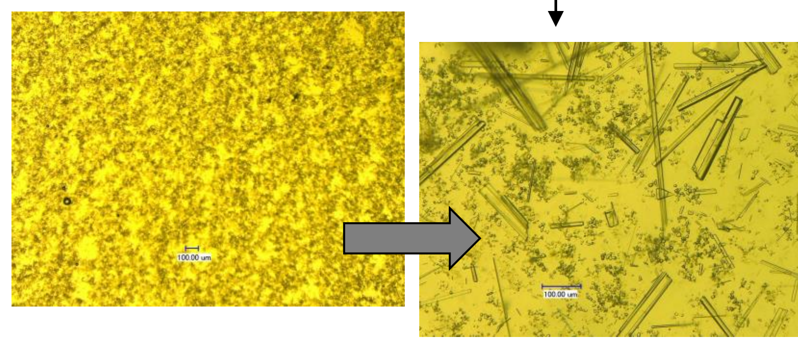
$CaCl_2$*2H_2O, 24h



Tetragonal

Na_2CO_3,

24 h   14 d



Orthorhombic

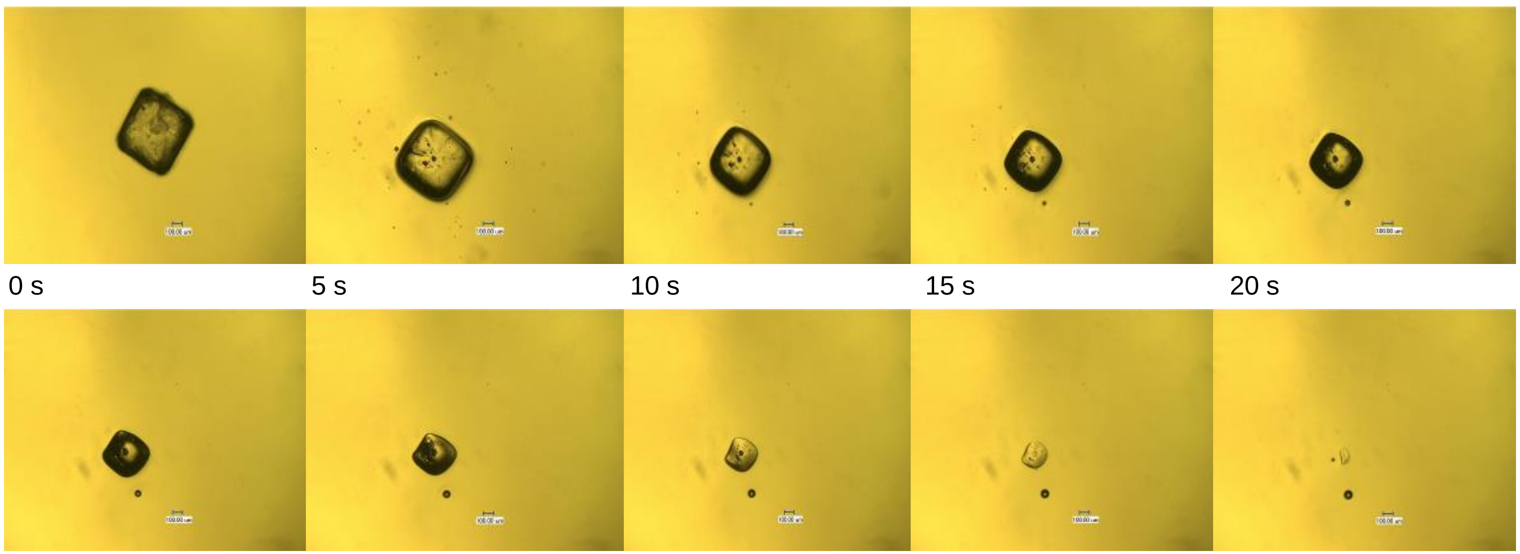
→ **type of salt has high impact on crystal outcome**

Müller, C., How to describe protein crystals correctly? –case study of lysozyme crystals-, Dissertation, Martin-Luther-Universität Halle-Wittenberg, 2012, http://141.48.65.178/hs/content/titleinfo/1177647.

dissolution of lysozyme crystals

dissolution behavior of NaCl

- known dissolution behavior of NaCl crystals in distilled water at room temperature



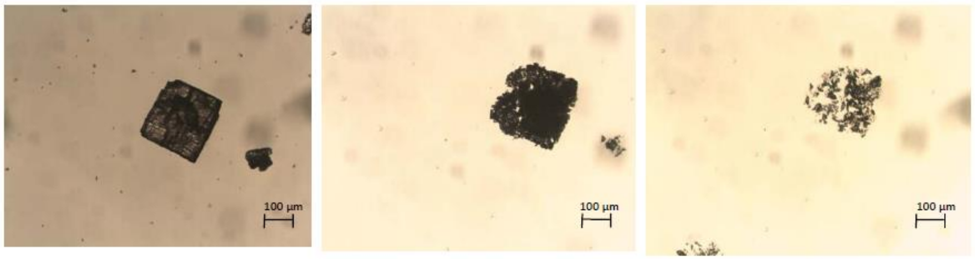
0 s 5 s 10 s 15 s 20 s

25 s 30 s 35 s 40 s 45 s

dissolution of lysozyme crystals

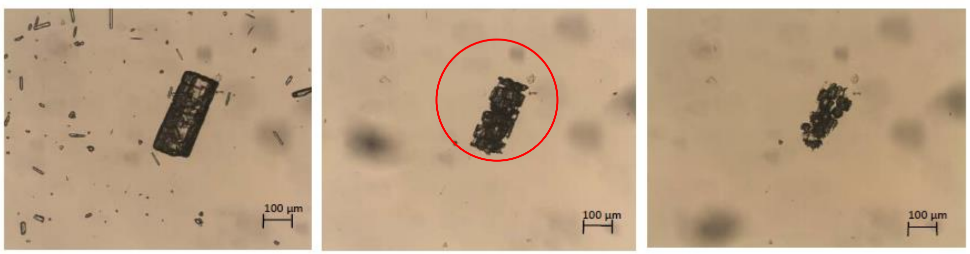
dissolution behavior

Tetragonal



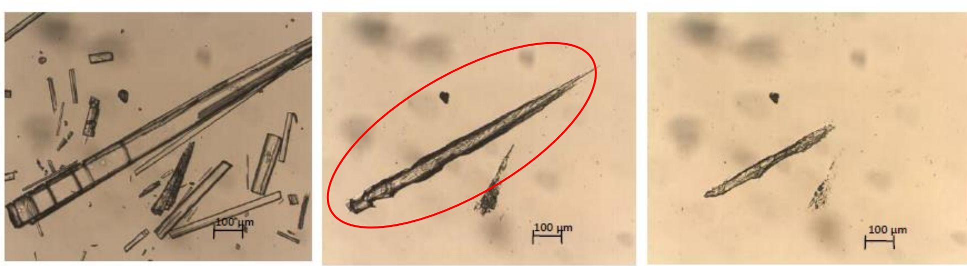
10 s | 10 h | 40 h

HTO



10 s | 2 h | 8 h

LTO



10s | 10h | 18h

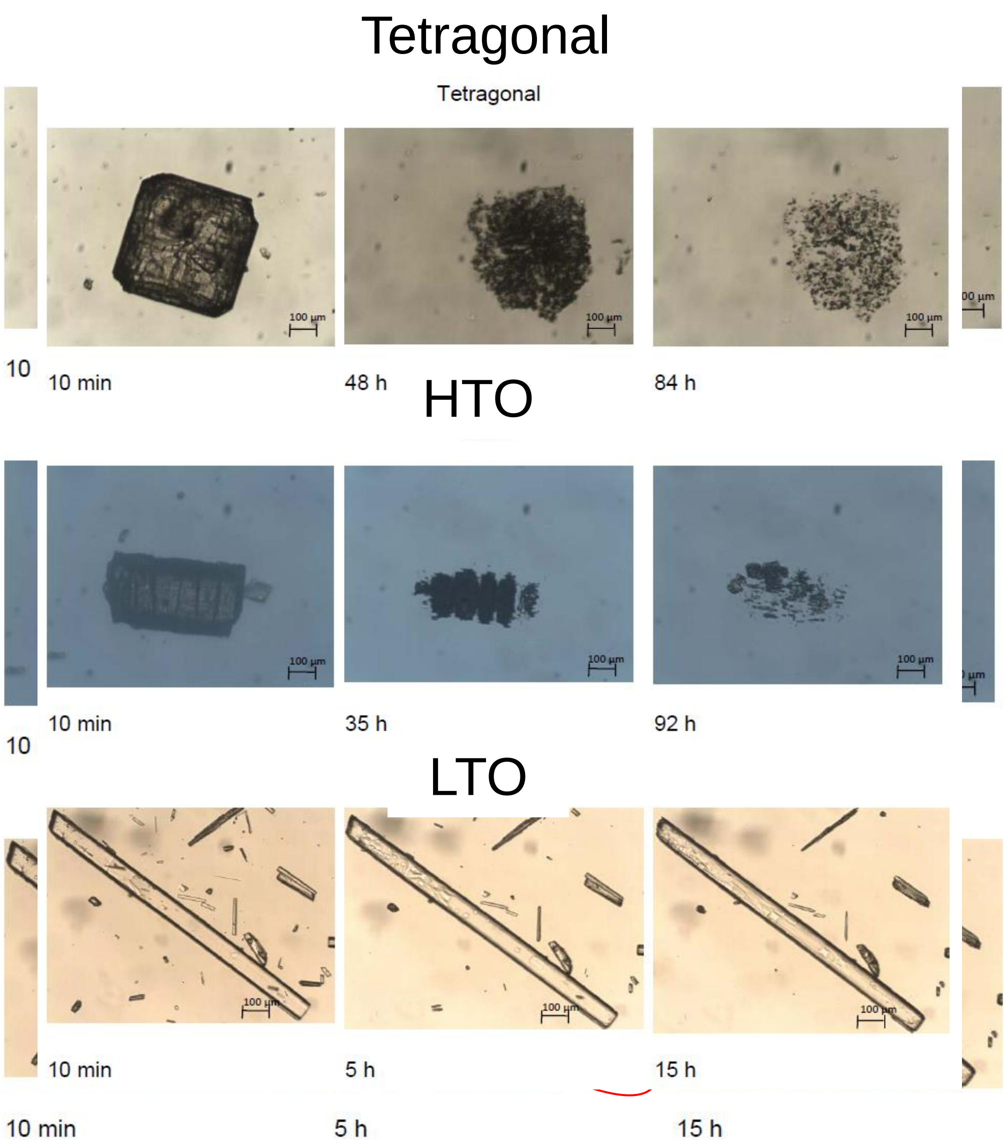
→ fresh lysozyme crystals in sodium phosphate buffer pH8 + 2 wt% NaCl at 16 ˚C

→ HTO falls apart in irregular particles

→ LTO changes its dissolution behavior again – no fragments

dissolution of lysozyme crystals

dissolution phenomenon



→ fresh lysozyme in glycine buffer pH10 + 7 wt% NaCl at 16 °C

→ why?

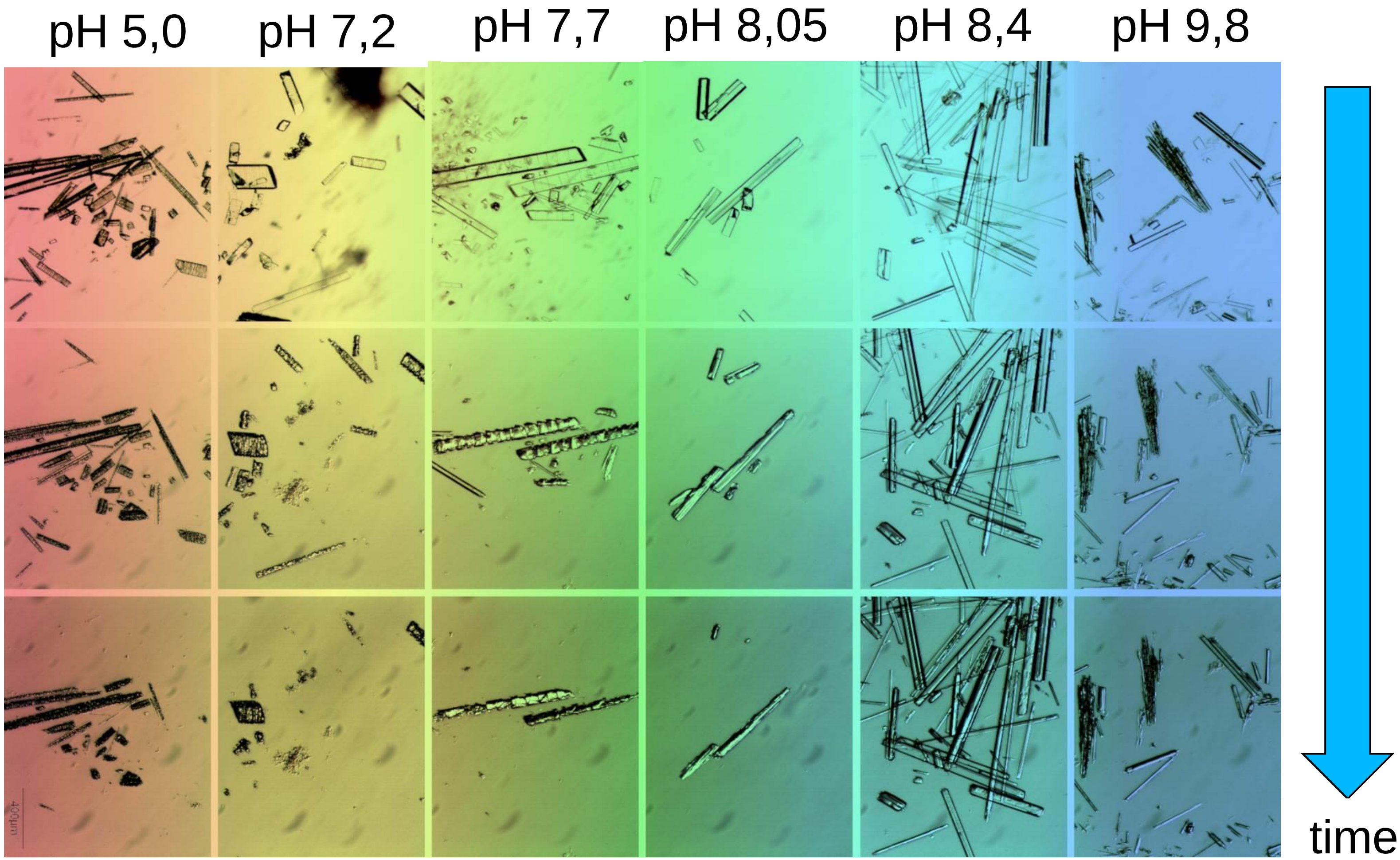
→ crystals fall apart during dissolution

→ changing dissolution condition changes the dissolution behavior of the HTO and LTO lysozyme crystals

→ HTO changes its dissolution behavior to “bar” shape fragments

→ LTO seems to not dissolve

dissolution behaviour versus pH-Value



Summary

- It is clear that the established terms form industrial crystallization do not fit the phenomena to be found in protein crystallization!

- Having a look at the dissolution phenomena shows quite clearly that there are not well considered phenomena existing.

Thank you for your attention!