Physical Basis of Steam-Sterilization

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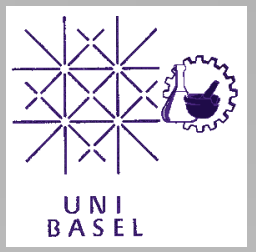
Water is a Solvent with unique Properties:



- Density of ice is lower than for liquid water
- High melting T_m and boiling temp. T_b at atmos.press.
- High amount of energy needed for evaporation of H_2O

$\Rightarrow$ **Life exists only due to these unique properties**

Water is unique: Typical Data I

H_2O with Molecular weight M = 18 compared to $C_3 H_6 S$ with Molecular weight 74 and with an *identical* dipole moment of μ = 1.85 Debye :

- **H_2O:** T_b = 100 °C at atmospheric pressure
- **$C_3 H_6 S$:** T_b = 14 °C at atmospheric pressure

$\Rightarrow$ ***Unique properties not due to dipole moment***

Water is unique: Typical Data II

	H_2O	H_2S	H_2Se	H_2Te
Molecular Weight M	**18**	**34**	**81**	**130**
Melting Temp T_m [°C]	**0**	**- 85,6**	**- 65,4**	**- 51**
Boiling Temp T_b [°C]	**100**	**- 60,8**	**- 41,5**	**- 3,8**

⇒ ***Unique properties due to Hydrogen Bonding?***

Water is unique: Typical Data III

	H_2O	H_2S	H_2Se	H_2Te
Binding enthalpy H-X [kJ/mol]	**464,4**	**338,9**	**305,4**	**267,8**
Melting enthalpy [kJ/mol]	**6,0**	**2,4**	**2,5**	**4,1**
Evaporation enthalpy [kJ/mol]	**40,7**	**18,7**	**19,9**	**23,9**

⇒ *Unique properties of water as a liquid: is it a „polymer“?*

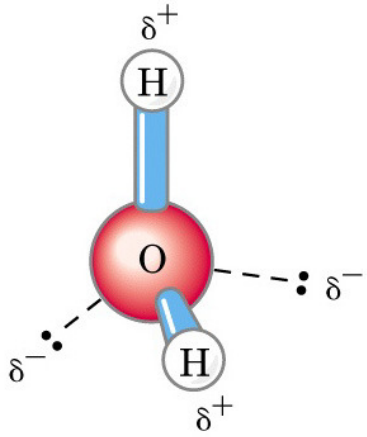
Water is unique: Structure

The *structure* of liquid water is *not very well known* and there is *no generally accepted theory* of liquid water:

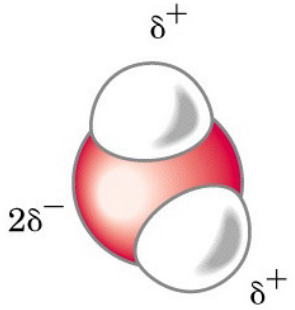
⇒ it is assumed that there is a *dynamic formation and dissolution of nanoclusters*, *i.e. nano-icebergs* in liquid water.

⇒ *Thus, liquid water has some „crystalline“ properties and is partly highly structured (see next slides).*

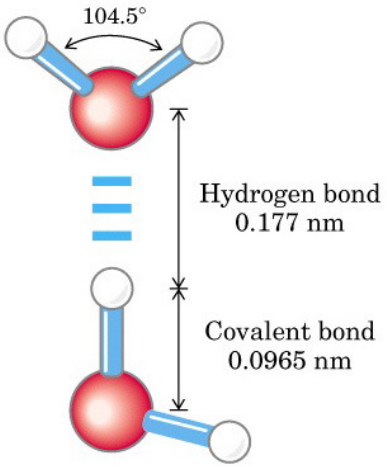
Water Structure



(a)



(b)



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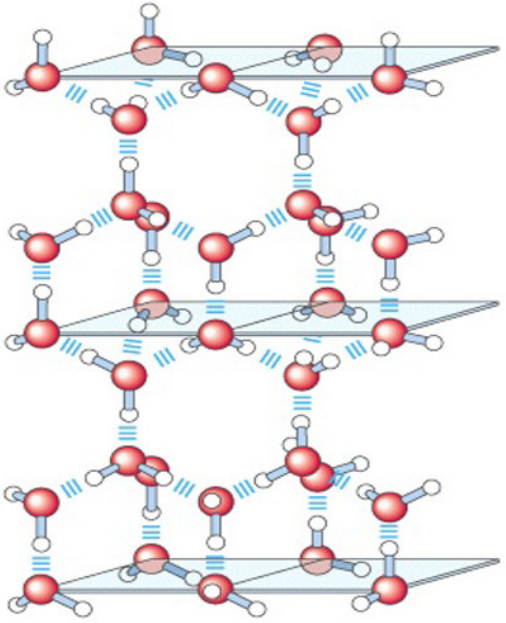
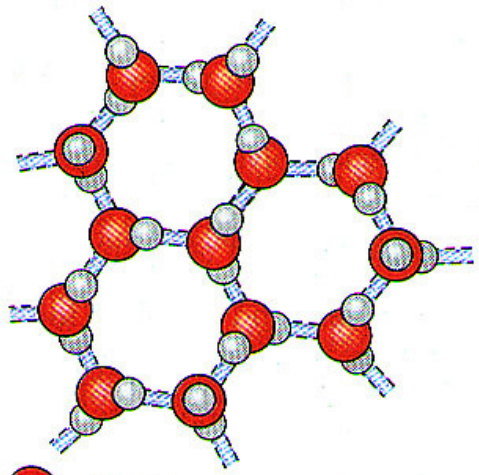


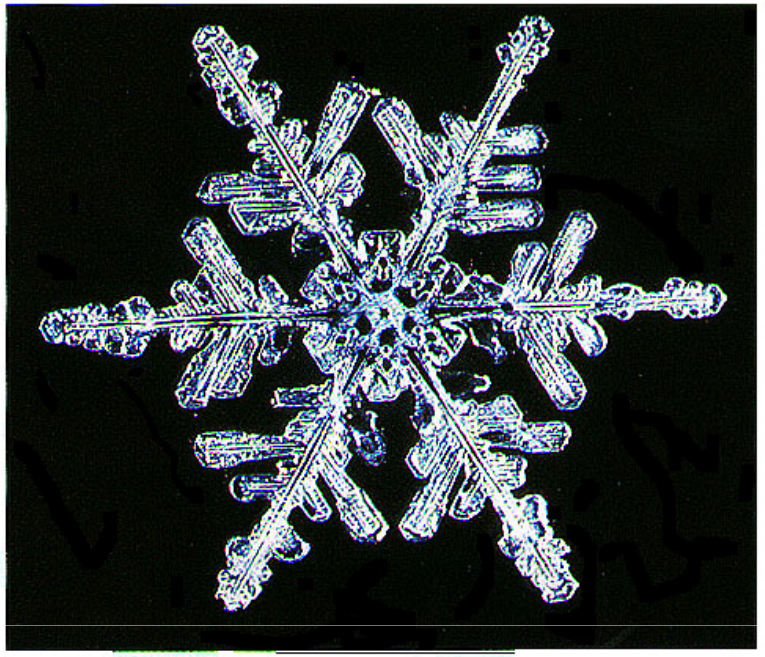


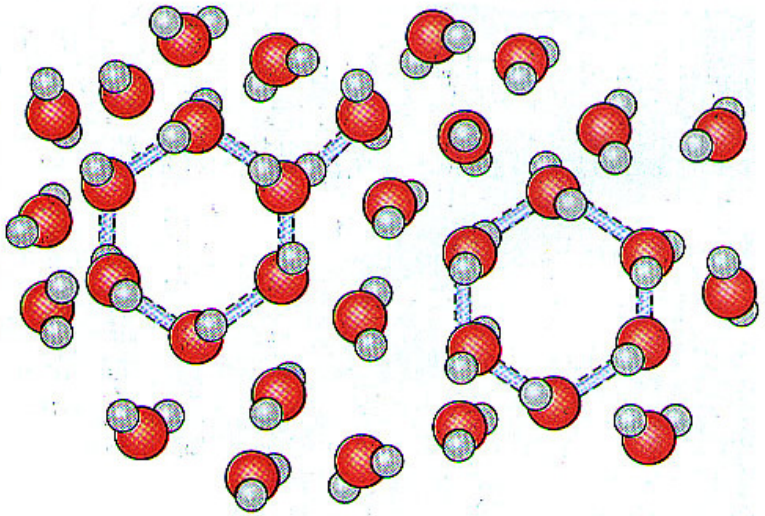
= Oxygen

= Hydrogen

= Water molecule

= Water molecule tipped so that one hydrogen is interacting with the plane above or below





Structure of Water: *Consequences*

The *structure* of water plays an important role in hydrophilic drug solutions (*solubilization* of drugs):

⇒ it is assumed that cosolvents and the drugs dissolved partly distroy the original water structure thus *changing the unique properties* of water [1,2]

1) A.Stengele, S.Rey and H. Leuenberger, A novel approach to the characterization of polar liquids part I: pure liquids, Int. J. of Pharm. 225, 123-134 (2001)
2) A.Stengele, S.Rey and H. Leuenberger, A novel approach to the characterization of polar liquids, part II: Hydrophilic Solutions, Int. J. of Pharm. 241, 231-240 (2002)

Unique Properties of Water:
Consequences for steam sterilization

- The *energy released during the condensation of the vapor is responsable for the inactivation of the microorganisms exposed for a sufficient period of time.*

- *The inactivation process follows a first order kinetics*

$$\frac{dN}{dt} = -k \cdot N$$

N = Number of microorganisms
t = time
k = kinetic constant

Unique Properties of Water:
Consequences for steam sterilization

- ***The inactivation process:***

$$\frac{dN}{dt} = -k \cdot N$$

dN = Number of microorganisms inactivated per unit time dt

k = kinetic constant

$$k = A \cdot e^{-\frac{E_a}{R \cdot T}}$$

The kinetic value k depends on the temperature T according to the law of Arrhenius.

Unique Properties of Water:
Consequences for steam sterilization

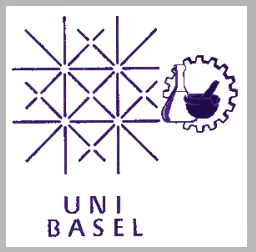
- ***The inactivation process:***

$$k = A \cdot e^{-\frac{E_a}{R \cdot T}}$$

E_a = (in)activation energy
R = Gas constant
A = Attempt frequency (unit t^{-1})

The kinetic value k (unit: t^{-1}) depends on the temperature T according to the law of Arrhenius.

Unique Properties of Water:
Consequences for steam sterilization



- It is *prerequisite* that the *pharmaceutical solutions* for steam sterilization are really *hydrophilic,* i.e. that *steam* can be *formed* and can *condensate* within the formulation.

- For an optimal heat transfer, i.e. an optimal antimicrobial treatment of surfaces of materials (containers etc.) to be sterilized it is a prerequisite that the autoclave contains only vapor and no air (check relationship temperature/pressure).

Unique Properties of Water:
Consequences for steam sterilization

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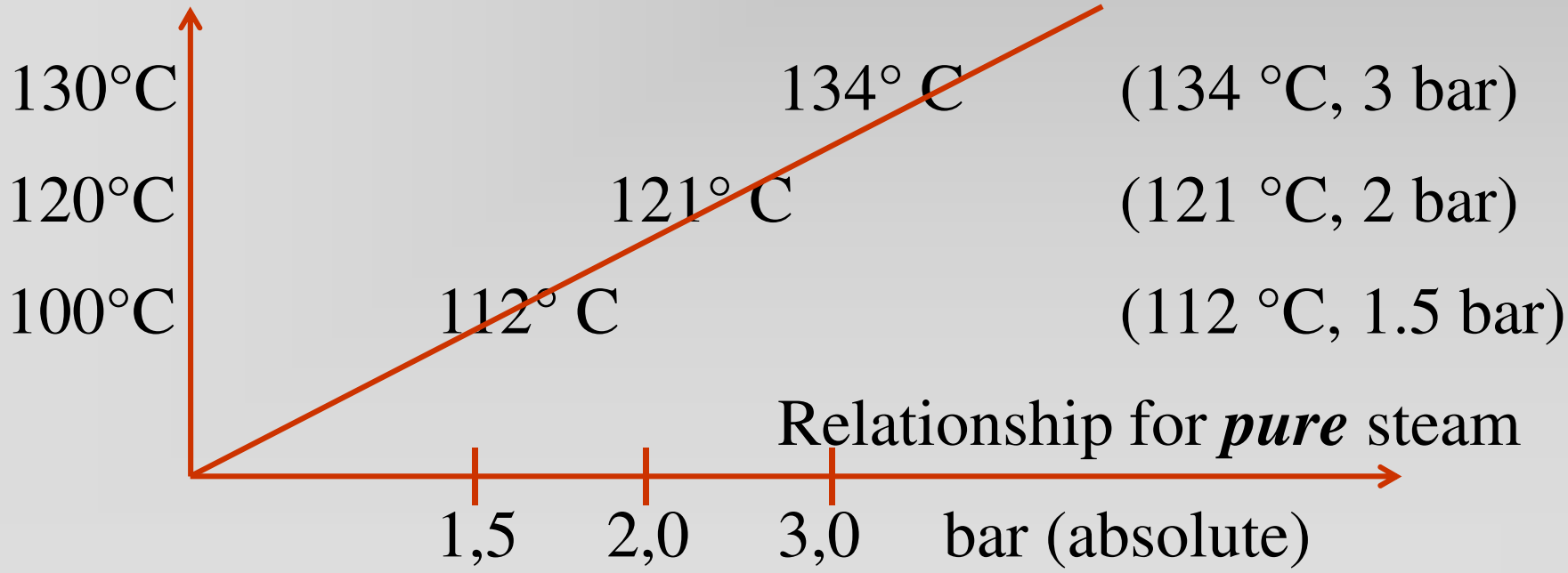
⇨ Do evacuate the autoclave before introducing steam

<u>Check:</u> Relationship between Temperature and Pressure



Unique Properties of Water:
Consequences for steam sterilization

⇨ Heat transfer of water vapor superior to hot dry air

Relationship between Temperature and Pressure can be used for in-process control and validation purposes:

In case that the system should contain air and not only pure steam the resultant pressure is higher !

⇨ Thus all partial pressures contribute to the total pressure measured (Law of Dalton) !

Unique Properties of Water:
Consequences for steam sterilization

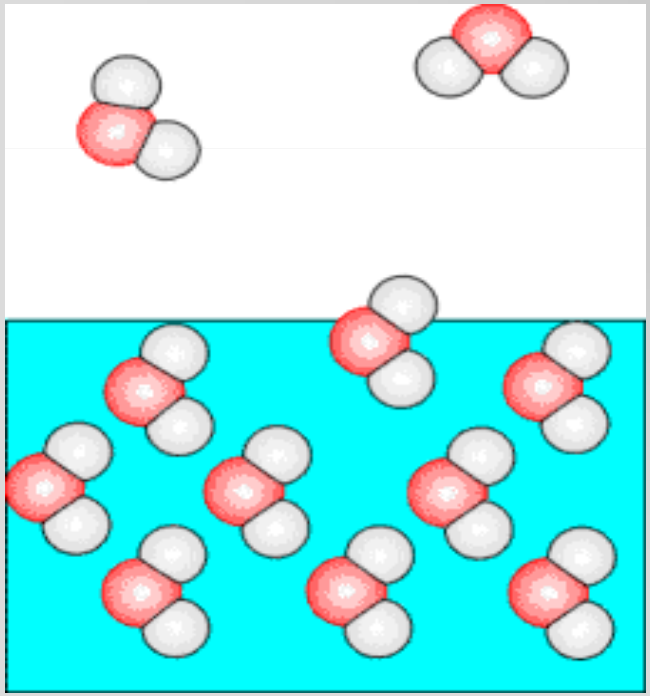
Relationship between Temperature and Pressure can be used for an in-process control of steam sterilization

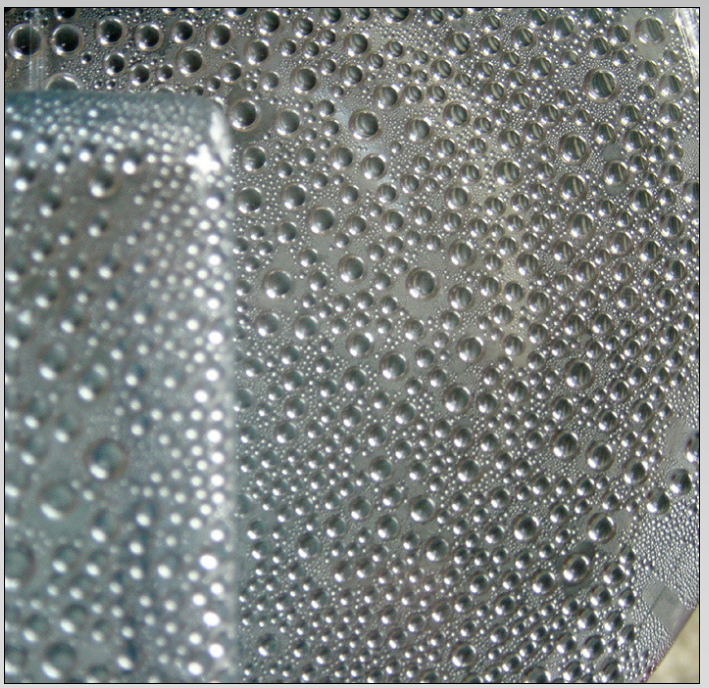
If the autoclave does not contain a vacuum pump for the evacuation of air, it is important that the air with its higher density can leave the autoclave through a valve at the bottom!

⇨ thus beakers to be sterilized should be put into the autoclave upside down, that the air is not trapped.

Steam Sterilization

Technical Remark: Vacuum facilitates evaporation and high pressure condensation

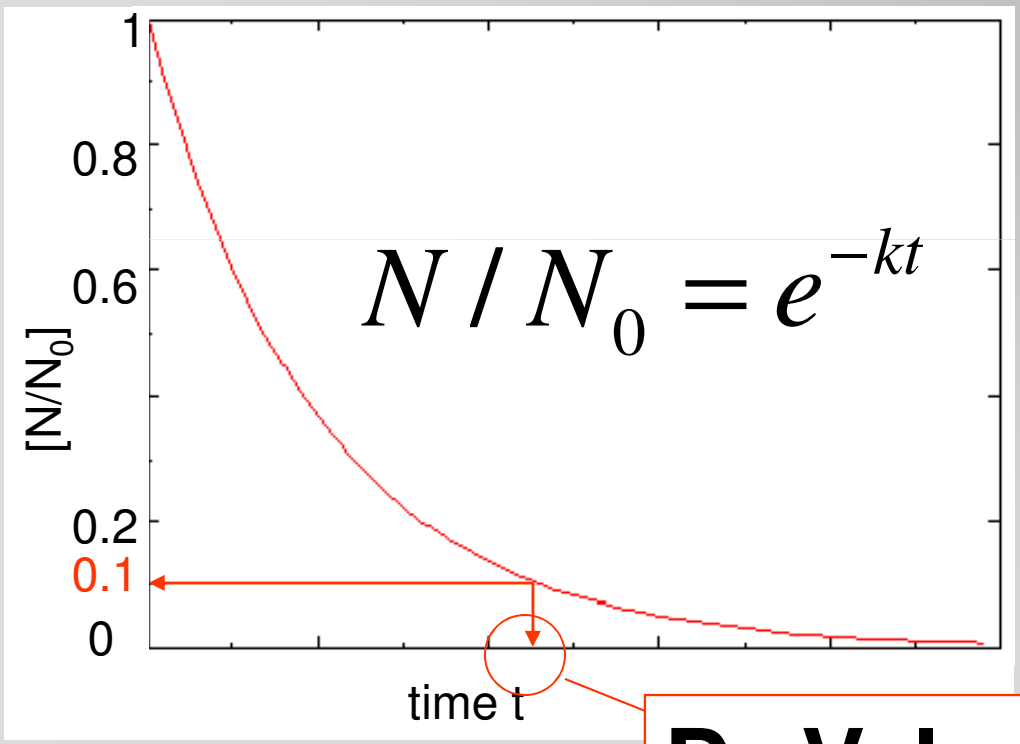




Physical Basis of Steam Sterilization

Inactivation of microorganisms

Exposure time D_T = „Decimal reduction time“



i.e. the number of microorganisms is reduced by a factor 0.1

i.e. there is a 90% reduction of N/N_0

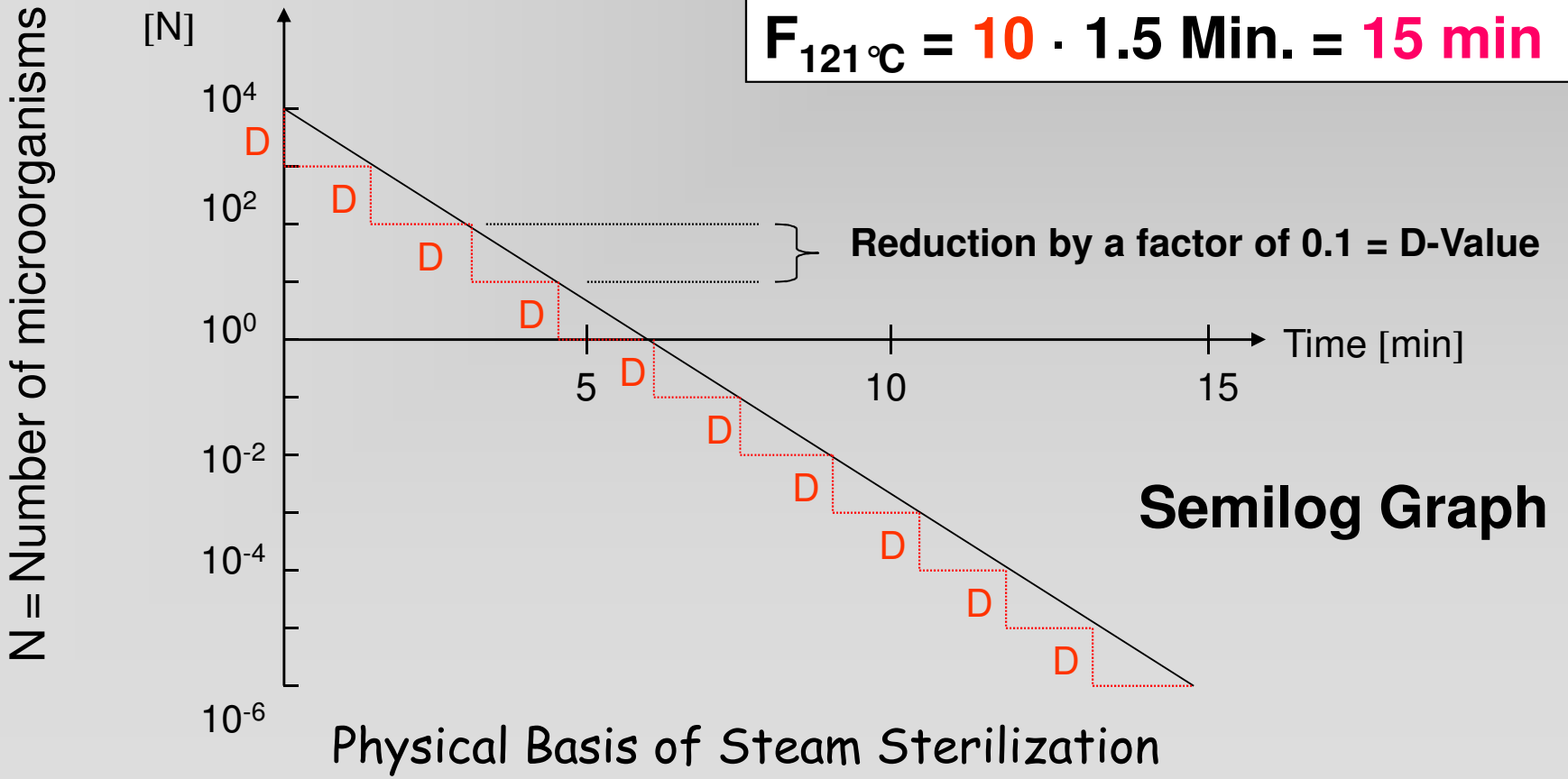
D_T value depends on exposure temperature T

D- and F-Value
The Overkill - Method N = 10^{-6}

$F = n\ D_T \Rightarrow$ for $N = 10^{-6} \Rightarrow F = ?, n = ?$

Bacillus Stearothermophylus D_T = 1.5 min at T = 121 °C, thus n = 10

$F_{121\,°C}$ = 10 · 1.5 Min. = 15 min



Physical Basis of Steam Sterilization

F-Value: an excellent concept
N = 10-6

F = n D for N = 10-6 $\Rightarrow$ F = ?, n = ?

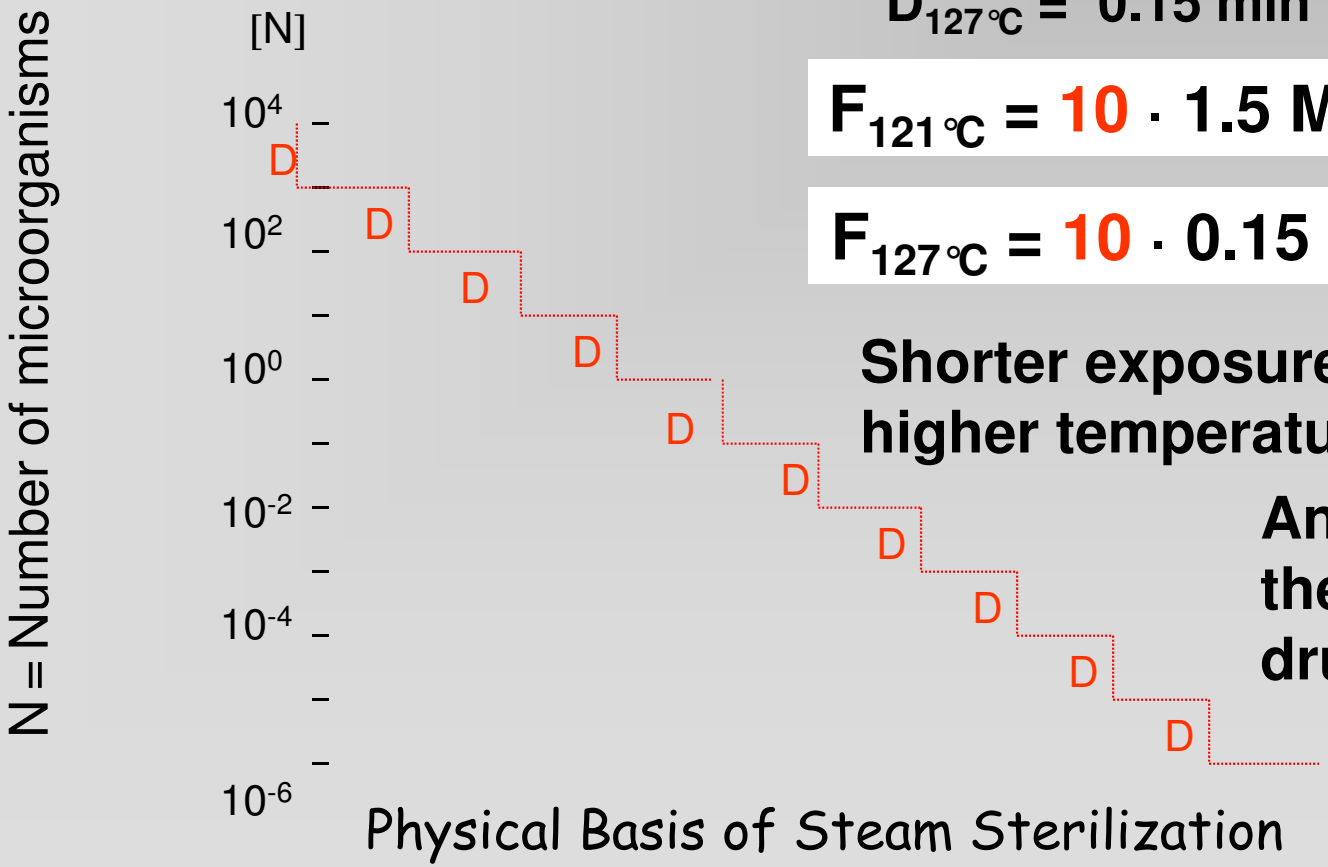
Bacillus Stearothermophylus

$D_{121\,°C}$ = 1.5 min n = 10

$D_{127\,°C}$ = 0.15 min n = 10

$F_{121\,°C} = 10 \cdot 1.5$ Min. = 15 min

$F_{127\,°C} = 10 \cdot 0.15$ Min. = 1.5 min



Shorter exposure time at a higher temperature !

An advantage for thermosensitive drugs?

$F_T = n D_T$

Physical Basis of Steam Sterilization

Inactivation of *microorganisms* versus *Degradation* of the drug *substance*:

Both inactivation and degradation processes follow the law of Arrhenius !

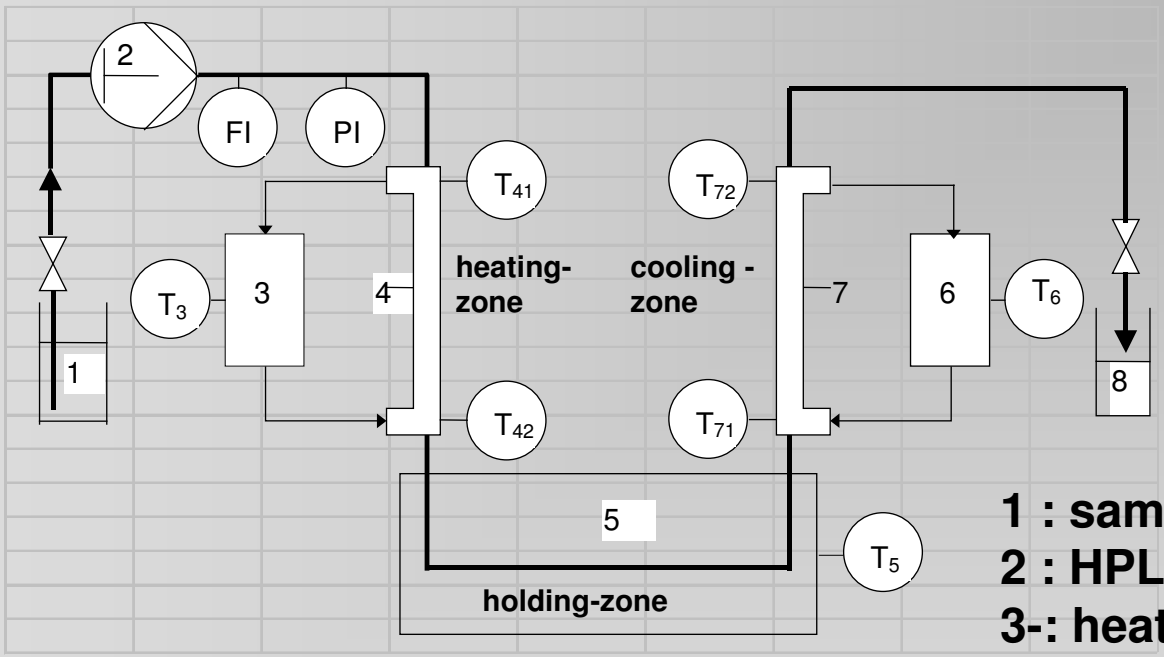
$$k = A \cdot e^{-\frac{E_a}{R \cdot T}}$$

It is evident that the k values are different for both processes and depend on the respective E_a and A

For such a situation a continuous short-time high temperature sterilization process was developed (PhD-Thesis*)

A. Mann[1,2]*, M. Kiefer[2], H. Leuenberger[1] J.Pharm. Sci. 90, 275-287(2001)
[1] University of Basel, [2] Novartis Pharma Ltd., Basel

Continuous short-time high temperature sterilization process

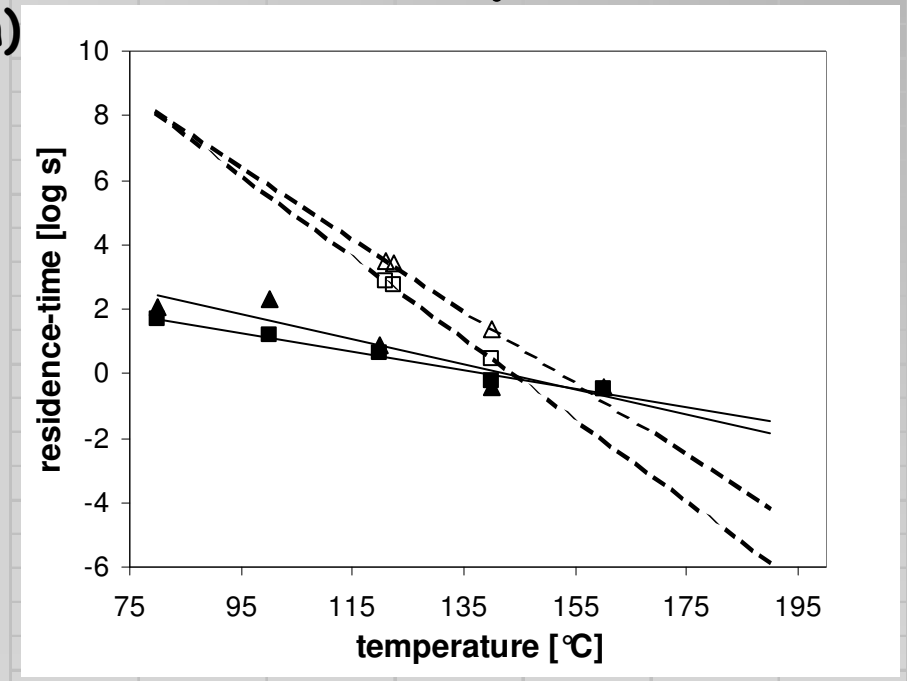


1 : sample stirring/cooling
2 : HPLC pump
3-: heat-/cool-ing system
8 : sample collection

Experimental set-up according to PhD Thesis Angelika Mann*
*** actual address: Novartis Pharma Ltd., Basel, Switzerland**

Continuous high temperature Short-time sterilization process

(Symbol=Formulation)



unfilled symbols:
inactivation

filled symbols:
drug degradation

Residence time for optimal sterilization process at T > 135°C;
Process parameters causing 12 log reduction of B. stearothermophilus spores (*dotted lines*) and causing < 0.5% drug degradation (*full lines*).

A. Mann[1,2]*, M. Kiefer[2], H. Leuenberger[1] J.Pharm. Sci. 90, 275-287(2001)
[1] University of Basel, [2] Novartis Pharma Ltd., Basel

Acknowledgements

Marijke Walter PhD student, University of Basel

Christina Erb University of Basel

Angelika Mann PhD, Novartis Pharma Ltd. Basel

Thank you for your Attention

Hans Leuenberger
Institute of Pharmaceutical Technology
University of Basel, Switzerland