From sticky mucus to Probing our Past: Aspects and problems of the Biotechnological use of Macromolecules

Datum/Zeit	Veranstaltungsort	Thema
Mi, 30.06.2010 12.15-13.45	SR 309 Carl-Zeiss-Str. 3	***Macromolecules as BioPharma mucoadhesives***
Do, 01.07.2010 08.15-09.45	SR 308 Carl-Zeiss-Str. 3	***Macromolecules as vaccines***
Do, 01.07.2010 13.15-14.45	HS Haus 1 August-Bebel-Str. 2	***Stability in response to Bioprocessing I. Thermal Processing, D, z and F values***
Fr, 02.07.2010 08.15-09.45	HS Haus 1 August-Bebel-Str. 2	***Stability in response to Bioprocessing II: Irradiation and freezing***
Fr, 02.07.2010 12.15-13.45	SR 307 Carl-Zeiss-Str. 3	***The use of non-recombining parts of the Y-chromosomal DNA and mitochondrial DNA as a probe into our past***

Food and Biopharma Processes imposing stresses on macromolecules:

- Thermal Processing
- Irradiation
- Freeze-thaw
- Spray drying
- Filtration
- Extrusion
- Lyophilisation

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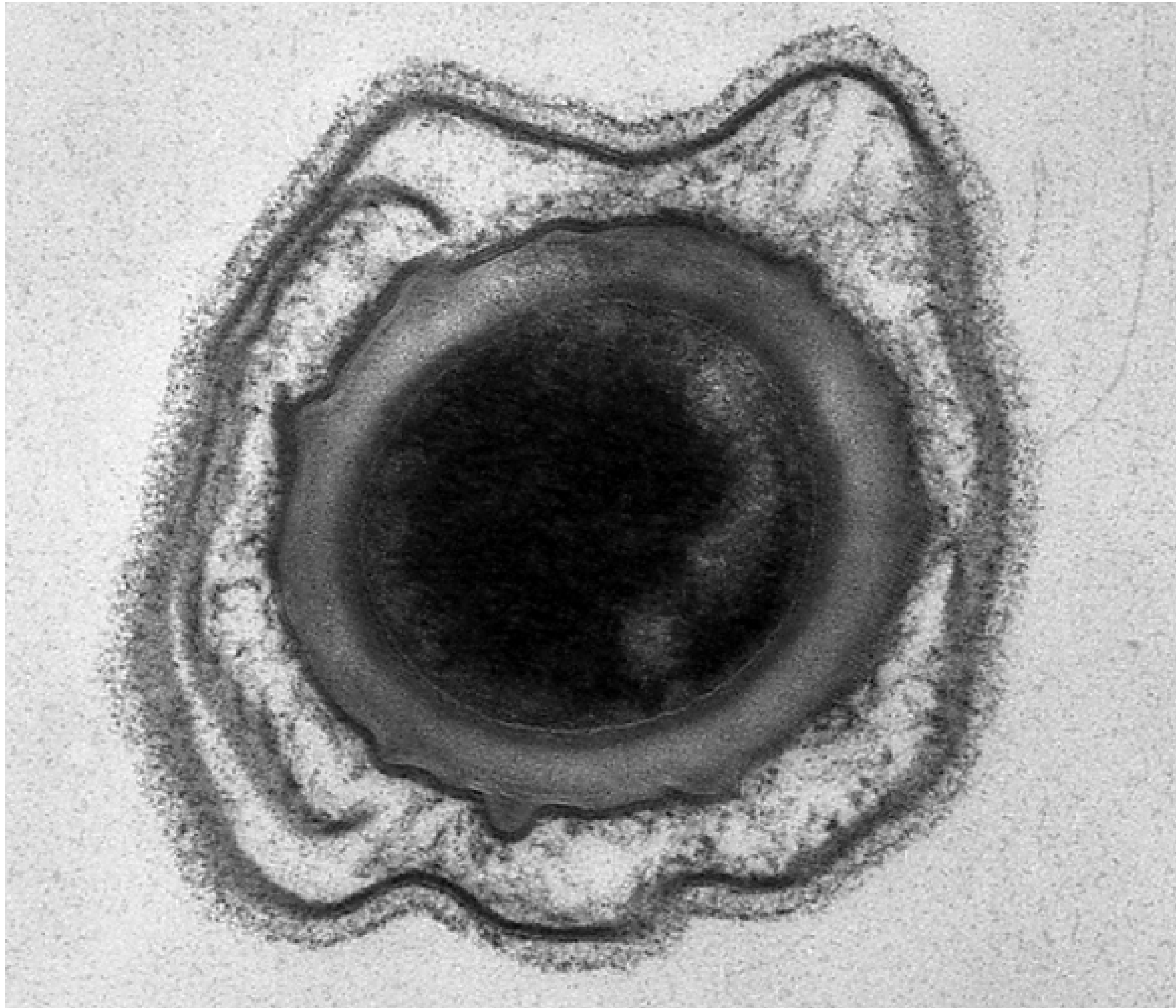
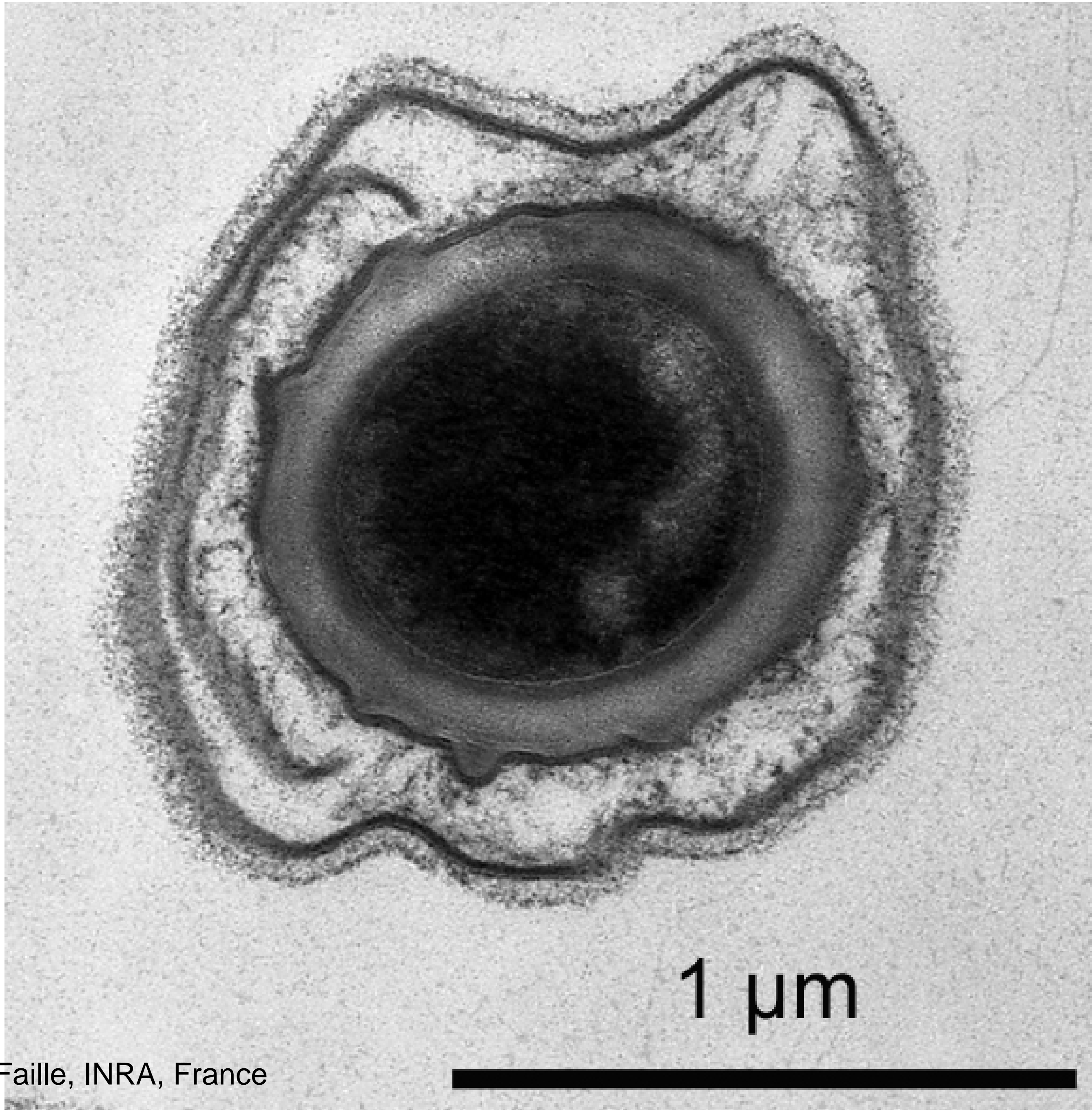
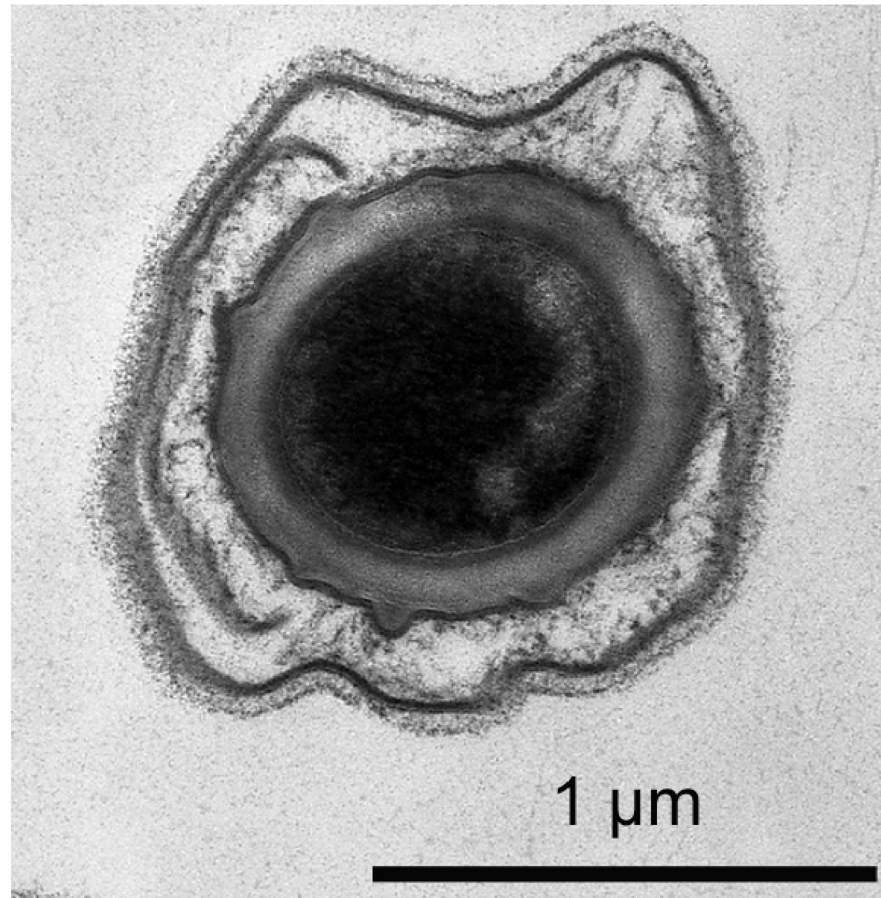




Courtesy of C. Faille, INRA, France

Stability in Response to Bioprocessing I: Thermal processing, D, z and F values



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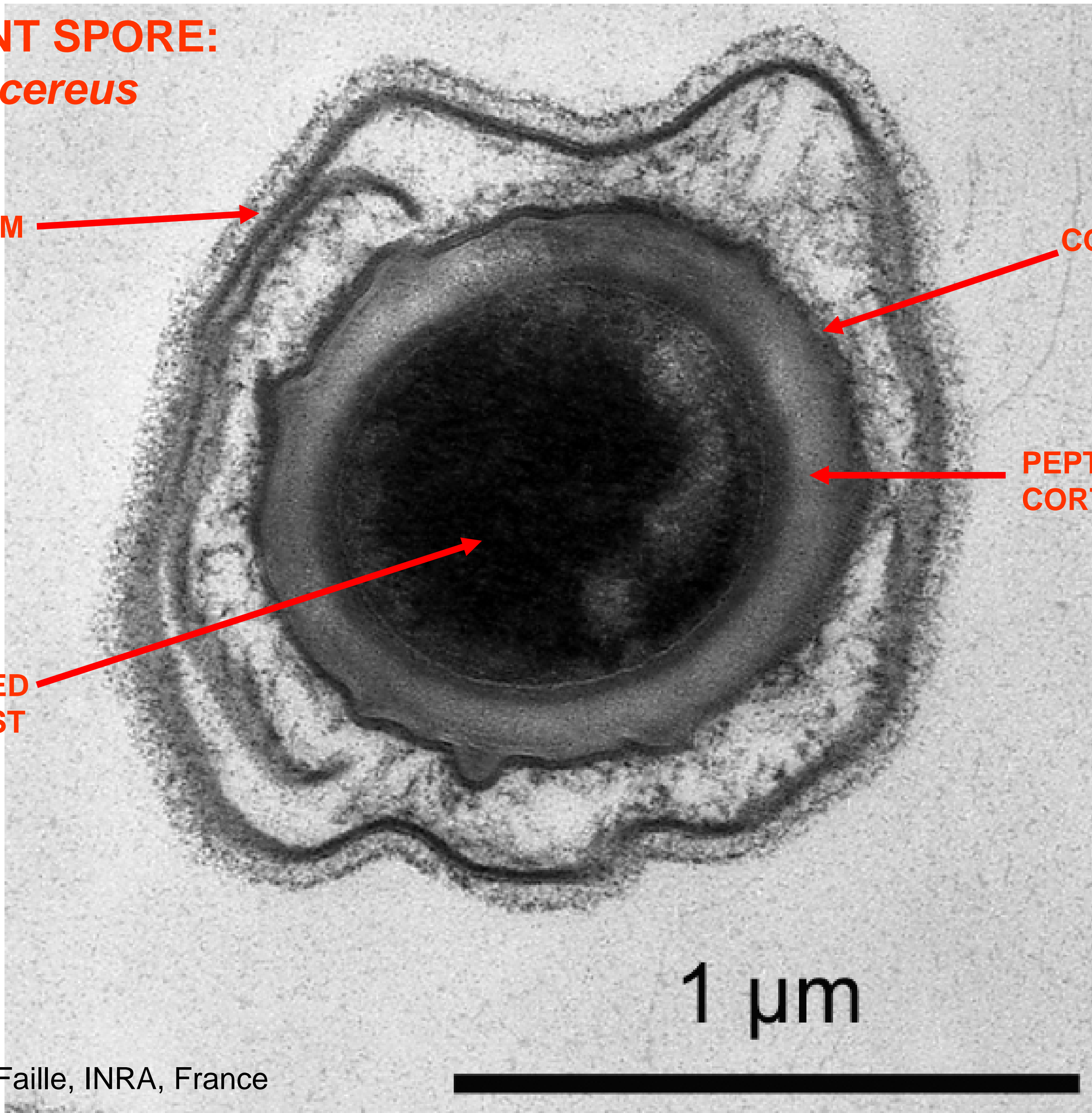

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- Most bacteria (*Salmonella*, *Listeria* etc) can be destroyed by pasteurisation, but the spore forming ones such as *Clostridium botulinium* require severe heat treatment at temperatures >100°C.
- Other bacterial spores may not be pathogenic but can cause spoilage to the foods
- Thermal processing is the most common form of treatment – but can also destroy important components of the food from small vitamins to large proteins and polysaccharides
- The goal of Thermal Processing is to make the food safe for the consumer but minimising the disruption of the food components

So this presentation is about how the Processing Industry goes about doing this, the criteria used for safety (based on D, z and F values) and the consequences for the food components

Theories for the dehydration and heat resistance of spores

1. Lewis, Snell & Burr: Contraction of the peptidoglycan cortex is responsible.
2. Gould & Dring (Unilever, Bedford UK): high osmotic activity and expansion of the cortex is responsible, brought about by the presence of many unshielded acidic groups.
3. Ellar (Cambridge): dehydration of the protoplast occurs at an earlier stage by osmotis of the mother cell before the formationof the cortex & that the rigid cortex maintains the dehydrated state until the spore is ready to germinate.effects of infection, such as release of endotoxin, do not occur.

So at the onset of germination Theory 1 predicts an initial large volume expansion, Theory 2 an initial contraction, Theory 3 no dramatic change. The relatively rapid method of ***Dynamic Light Scattering*** *was used to test for this, and showed that the Ellar theory was the more likely.*

Biochem. J. (1984) **220**, 117–123
Printed in Great Britain

Quasi-elastic light scattering studies on dormant and germinating *Bacillus subtilis* spores

S. E. HARDING and P. JOHNSON
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

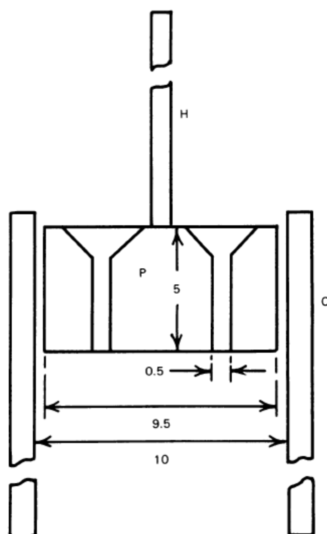

Fig. 1. *Rapid mixing plunger device used for introducing the germinant (cf. run 3 of Fig. 6)*
All dimensions are in mm.

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1. Drop in Optical Density (Absorbance) with time shows the progress of germination of the spores:

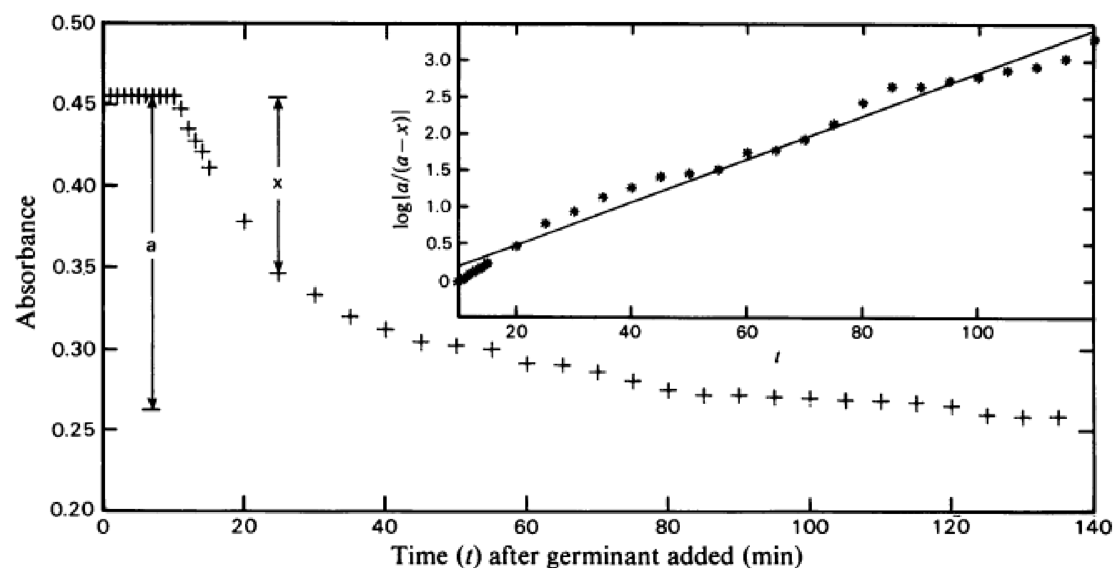

Fig. 5. *Plot of absorbance at 580nm as a function of time after addition of* L*-alanine to 0.02* M
Temperature was 35.0°C, spore concentration was 6×10^7/ml. Inset: first-order reaction plot; rate constant, $k_r = 0.029\,s^{-1}$.

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2. Progress of diffusion coefficient coefficient (a measure of size) with time shows no dramatic change in volume

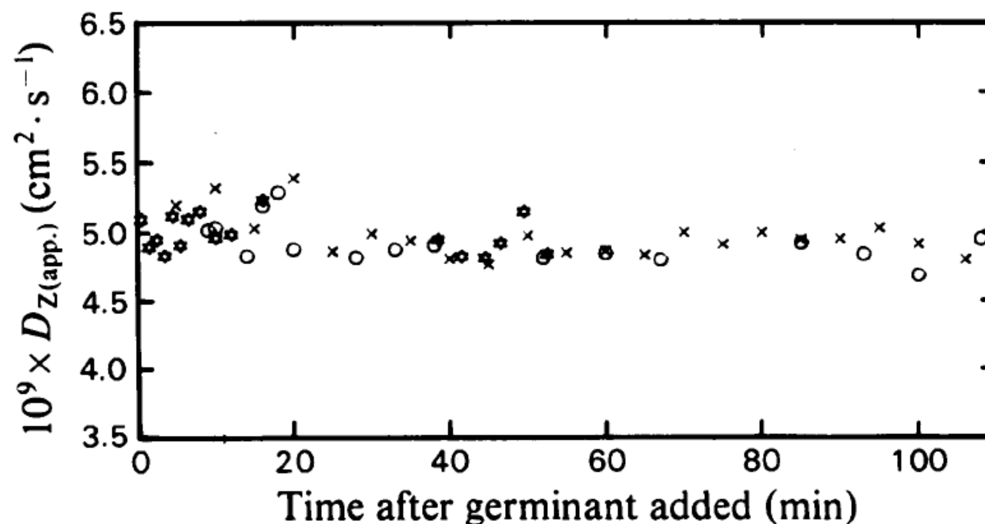
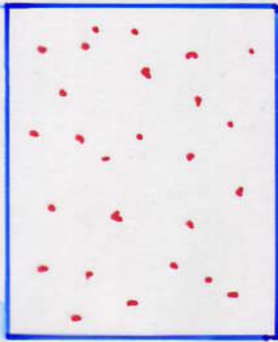


Fig. 6. *Plot of z-average apparent diffusion coefficient* $D_{Z(app.)}$ *at an angle, θ, of 90°, as a function of time after the addition of germinant*

Kinetics of bacterial (and nutrient) destruction

- Processing industry has to bring contamination down to a safe level based on agreed criteria but minimising damage to the nutrients
- Destruction of bacteria (and nutrients) follow a first order process
- Destruction rate dN/dt is proportional to N, the number of bacteria remaining
- Similar relations exist for molecular/macromolecular nutrients, except normally deal with number or weight concentrations, c (mol/ml) or C (g/ml)

Thermal Destruction at a Fixed Temperature T



Consider a homogeneous suspension of N bacteria / spores at time $t = t$, and N_0 at time $t = 0$

Destruction described by 1st order kinetics

$$\frac{dN}{dt} = -kN \quad (k \text{ is rate constant } s^{-1})$$

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

$$\int_{N_0}^{N} \frac{dN}{N} = -k\int_{0}^{t} dt$$

$$\therefore \ln N - \ln N_0 = -kt$$

$$\ln\left(\frac{N_0}{N}\right) = kt$$

Convert to base 10 :

$$2.303 \log_{10}\left(\frac{N_0}{N}\right) = kt$$

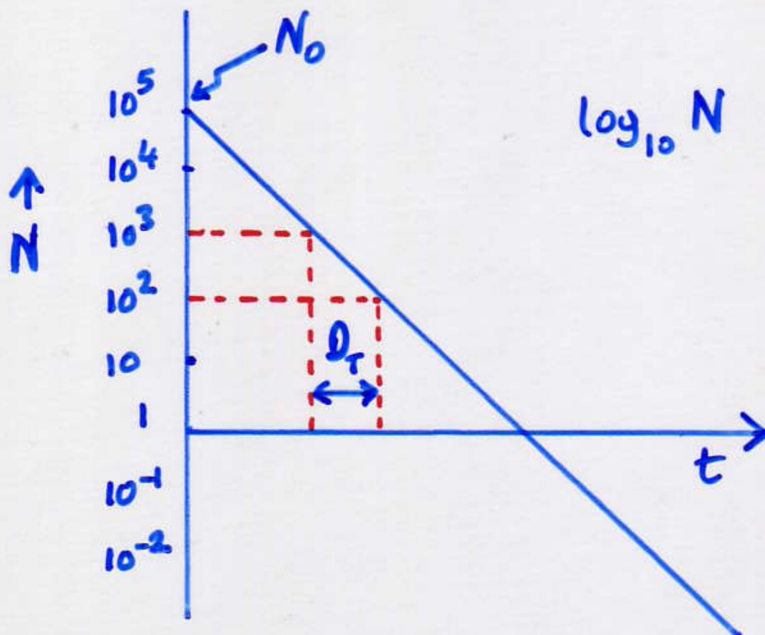
$$\therefore \log_{10}\left(\frac{N_0}{N}\right) = \frac{k}{2.303} t$$

or

$$\boxed{\log_{10}\left(\frac{N_0}{N}\right) = \frac{t}{D_T}}$$

$D_T = 2.303/k$ is the <u>DECIMAL REDUCTION TIME</u>

- the time (in sec or min) for the population to reduce by a factor of 10



$$\log_{10} N = -\frac{1}{D_T} t + \log_{10} N_0$$

Standard reference temperature $T = 121.1\,^\circ C$

e.g. $D_{121.1}$ for C. botulinum ~ 12 sec

Processing at a given FIXED Temperature

$$t = D_T \log_{10}\left(\frac{N_0}{N}\right)$$

$$\left[\frac{N}{N_0} = 10^{-t/D_T}\right]$$

Comments :

1. Time, t, required to get "safe" level N depends on N_0 + D_T

2. Mathematical probability of "no spores" $\rightarrow 0$ as $t \Rightarrow \infty$

3. The "12 D" concept - C. botulinum $[D_{121} \simeq 12$ sec. $12 D \simeq 144$ sec $]$

4. Other spores can have greater D values (up to 240 sec for D_{121})

Types of Spore

Cryophiles : grow only below ~ 20 °C

Mesophiles : optimum growth ~ 20 - 40 °C

e.g. *C. botulinum* (D_{121} ~ 12 sec)

Thermophiles : grow above ~ 40 °C

e.g. *B. stearothermophilus* (D_{121} ~ 240 sec)

Example

Microbiologists in a food company find contamination level by B. stearothermophilus ≈ 1 spore / gm. Avge. container has ≈ 570g. Quality control people require ~ 0.01 spore per container. What is process time, t, at 121°C? $\{D_{121} = 240 \text{ sec}\}$

≈ 570 spores / container

$$t = 240 . \log\left\{\frac{570}{0.01}\right\} = 1141 \text{ sec}$$

(19.0 min)

$$\equiv 4.75\, D_{121} .$$

Summary of Destruction at fixed temp.

$$\log_e\left(\frac{N_0}{N}\right) = kt$$

$$\log_{10}\left(\frac{N_0}{N}\right) = \frac{t}{D_T} \qquad (1)$$

where $D_T = \frac{2.303}{k}$

D_T ← 'decimal reduction time'

k ← reaction velocity constant

$D_{121.1}$ or "D_0"

2 useful forms of (1)

$$t = D_T \log_{10}\left(\frac{N_0}{N}\right)$$

$$\frac{N}{N_0} = 10^{-t/D_T}$$

nb D_{121} max for C. botulinum
≈ 12 secs

pH OF FOOD PRODUCTS

Food	pH	
Lemons	2·3 – 2·6	LOW RISK
Apples	3·0 – 3·3	
Strawberries	3·3 – 3·4	
Apricots	3·7 – 3·8	
Yoghurt	4·0 – 4·5	MEDIUM RISK
White cheese	4·0 – 4·5	
Beer	4·1 – 4·3	
Potatoes	5·4 – 5·8	HIGH RISK.
Meats	5·5 – 6·5	
Peas	6·1 – 6·3	
Milk	6·5 – 6·7	

$F_{121.1}$ VALUES

In specifying the degree of sterilization or "Lethality" of a thermal process, the Bioprocessing Industry rarely uses (N_0/N) values,

but rather $F_{121.1}$ values

The $F_{121.1}$ value is the equivalent process time at a constant temp. 121.1°C to give the same sterilization (N_0/N) to a process whose temperature is not necessarily 121.1°C & not necessarily constant

$$F_{121.1} = D_{121.1} \cdot \log_{10}\left(\frac{N_0}{N}\right)$$

So for a "12D" process (C. botulinum)

$F_{121.1} = 12 \times 12 = 144$ sec

or a "5D" process (B. stearothermophilus)

$F_{121.1} = 5 \times 240 = 1200$ sec

WHAT'S THE BEST TEMPERATURE FOR A THERMAL PROCESS? : Z VALUES

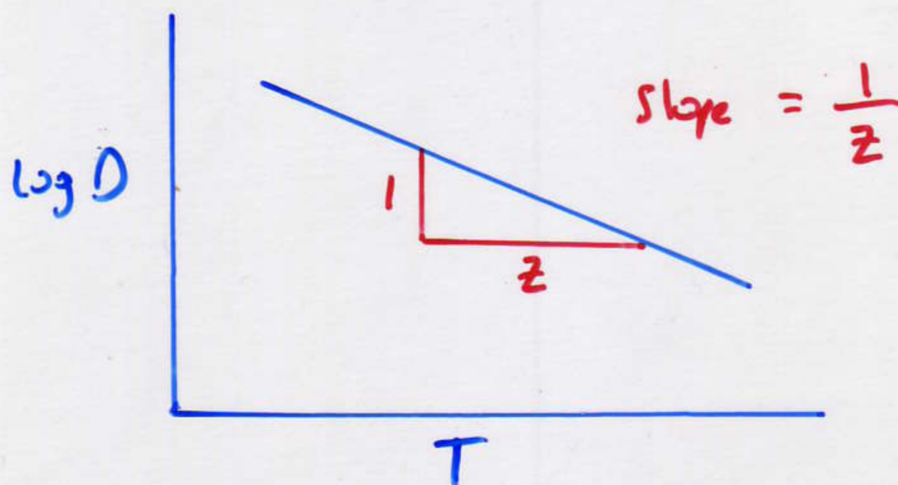
i.e. for destroying the spores without doing too much damage to the food itself - proteins, polysaccharides, vitamins etc.

The KEY lies in how D varies with Temp for spores compared with nutrients.

Over a limited range of temp. to a good approximation

$$\log_{10} D \propto -T$$

- known as Bigelow's approximation



C. botulinum $z \sim 10^\circ C$

Thiamine $z \sim 30^\circ C$

NOTE THE DIFFERENCE!

DIFFERENTIALS IN Z - VALUES

Long process times at lower temperatures

or Short process times at higher temps. ?

Thiamin : $Z = 26.3\,^\circ C$

Clostridium botulinum : $Z \simeq 10\,^\circ C$

$$\log\left(\frac{N_0}{N}\right) = \frac{t}{D_T}$$

BACTERIA

$$t = D_T \log_{10}\left(\frac{N_0}{N}\right)$$

$$\log t = \log D_T + \log\left[\log\left(\frac{N_0}{N}\right)\right]$$

NUTRIENTS

$$t = D_T \log_{10}\left(\frac{C_0}{C}\right)$$

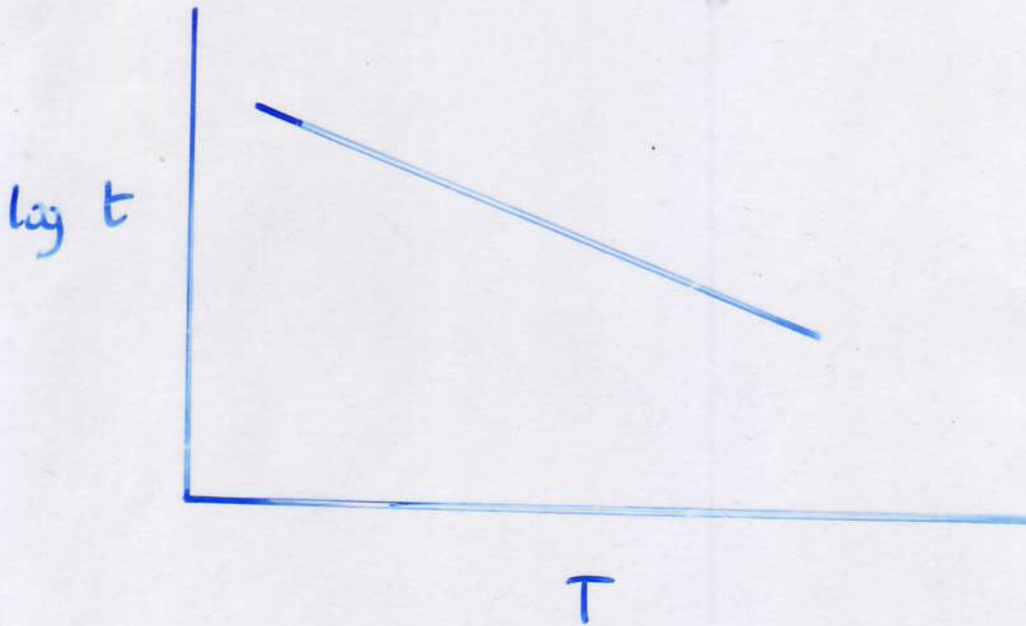
$$\log t = \log D_T + \log\left[\log\left(\frac{C_0}{C}\right)\right]$$

Now, using Bigelow's approximation

$$\log D_T \propto - T$$

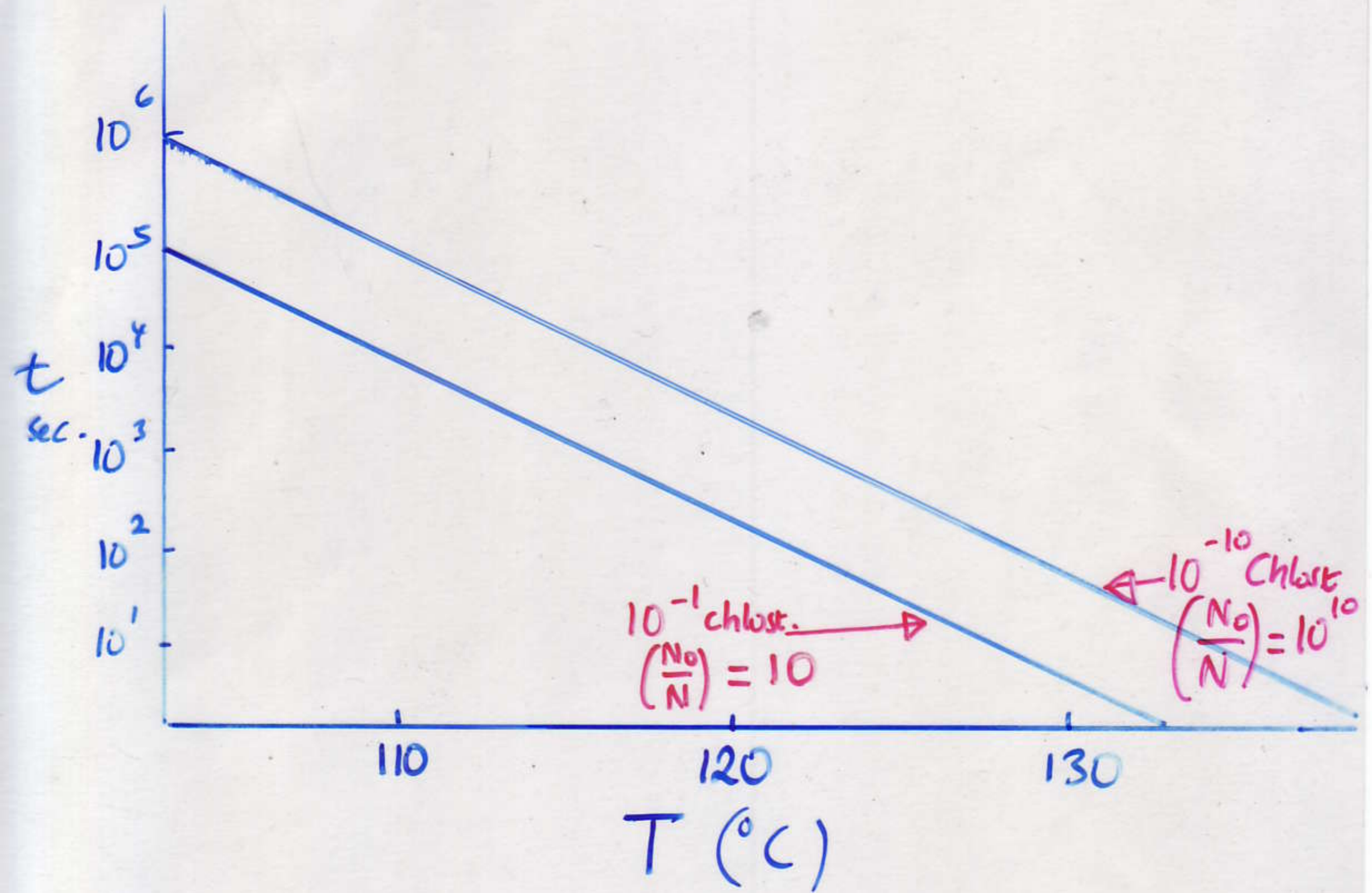
$\therefore$ For a fixed $\frac{C_0}{C}$ or $\frac{N_0}{N}$

$$\boxed{\log t \propto - T}$$



DIFFERENTIALS IN Z-VALUES

- choosing an appropriate temperature



DIFFERENTIALS IN Z-VALUES

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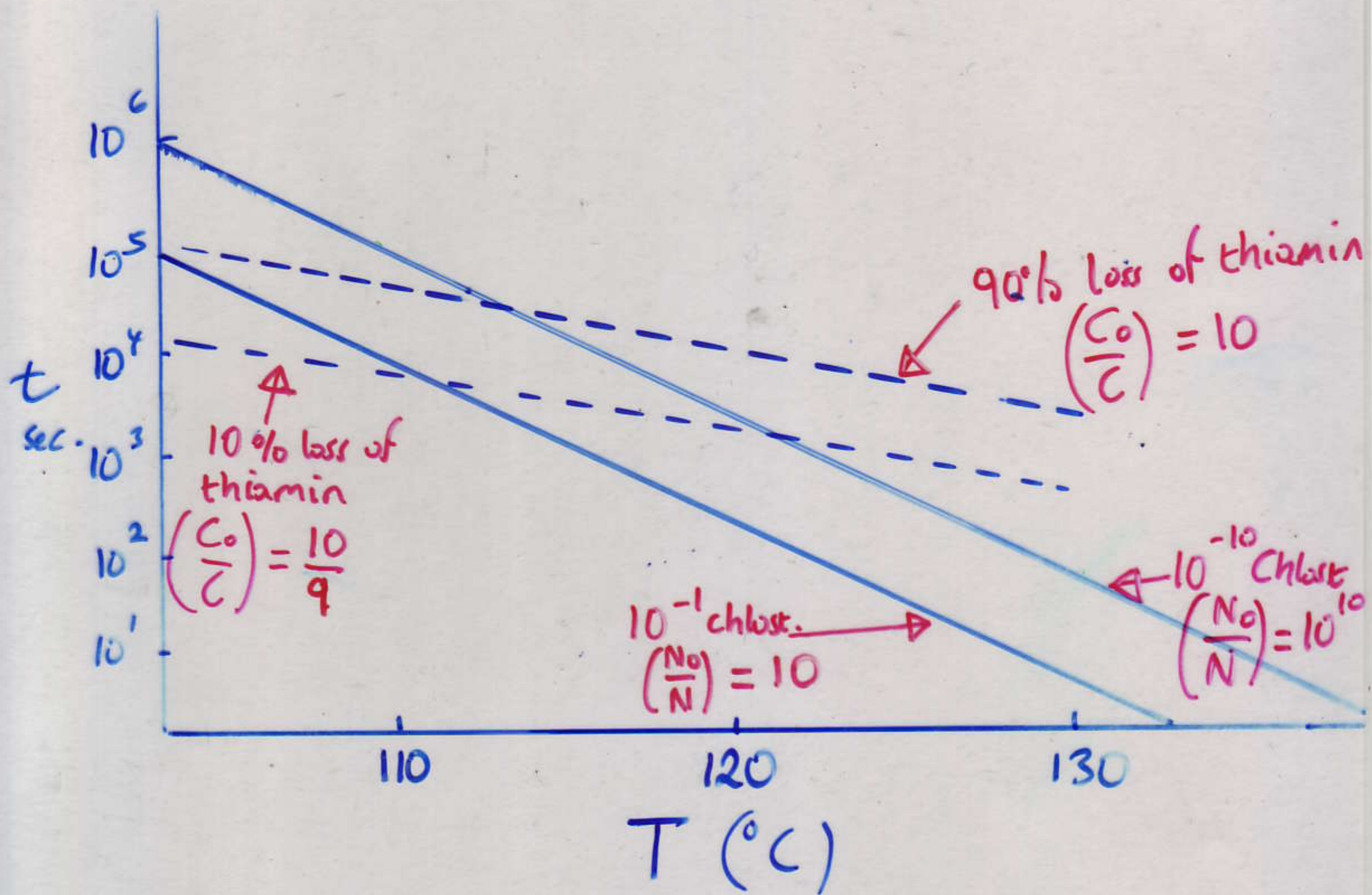
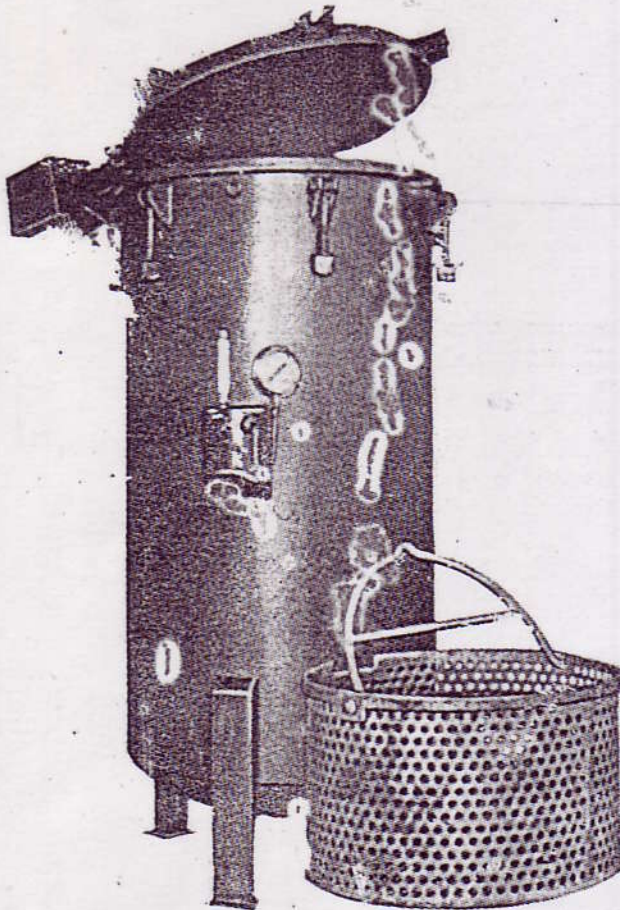


"BATCH RETORT"



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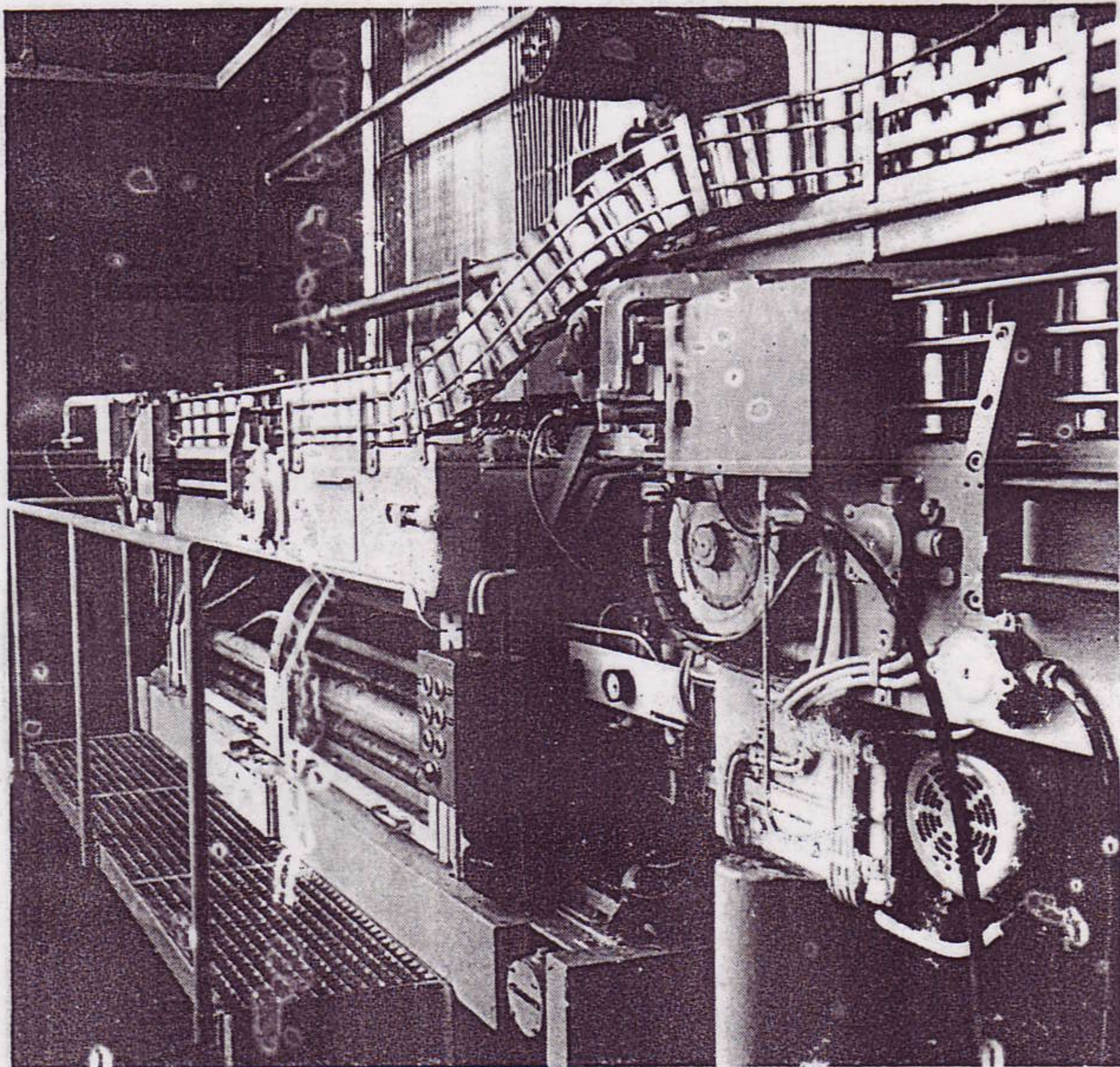
"HYDROSTAT"



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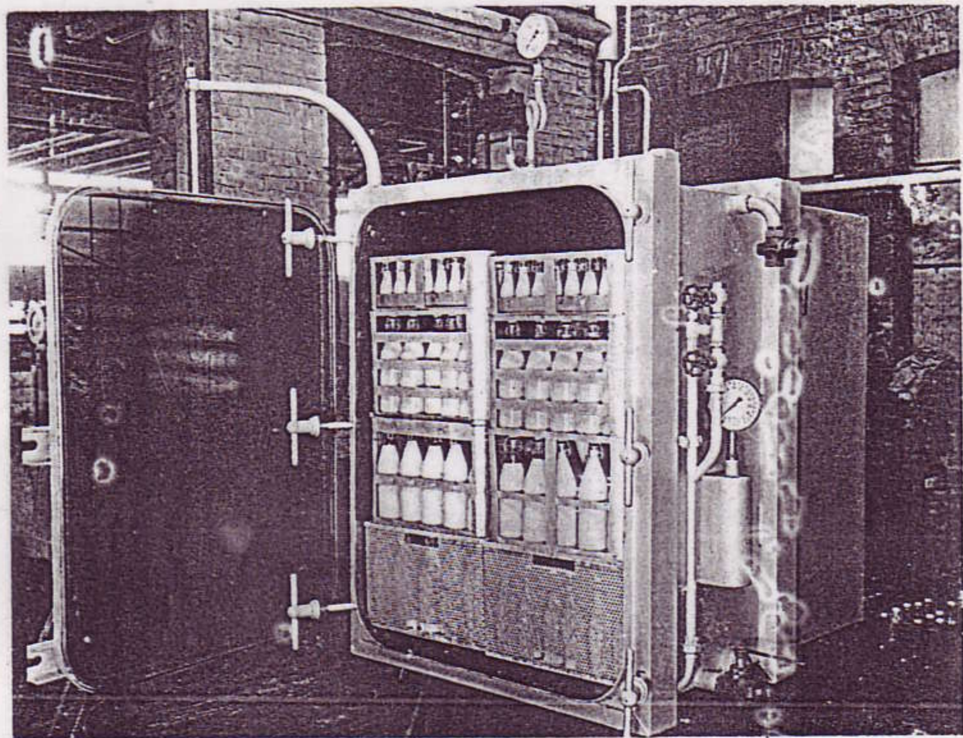
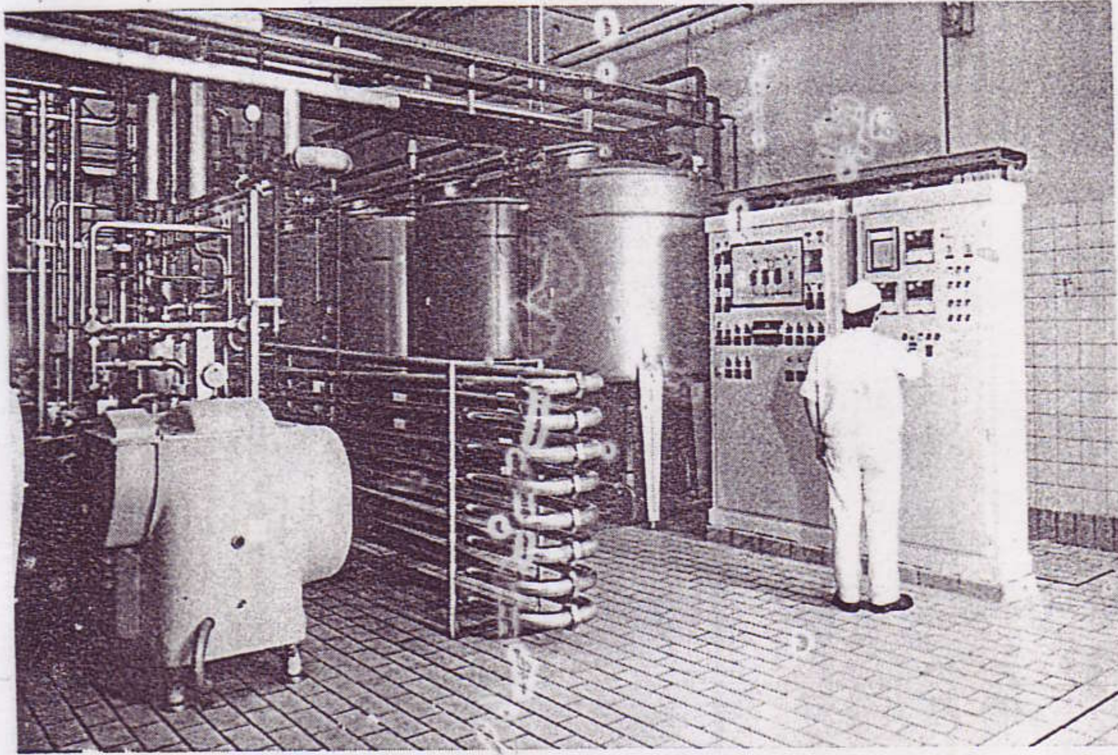
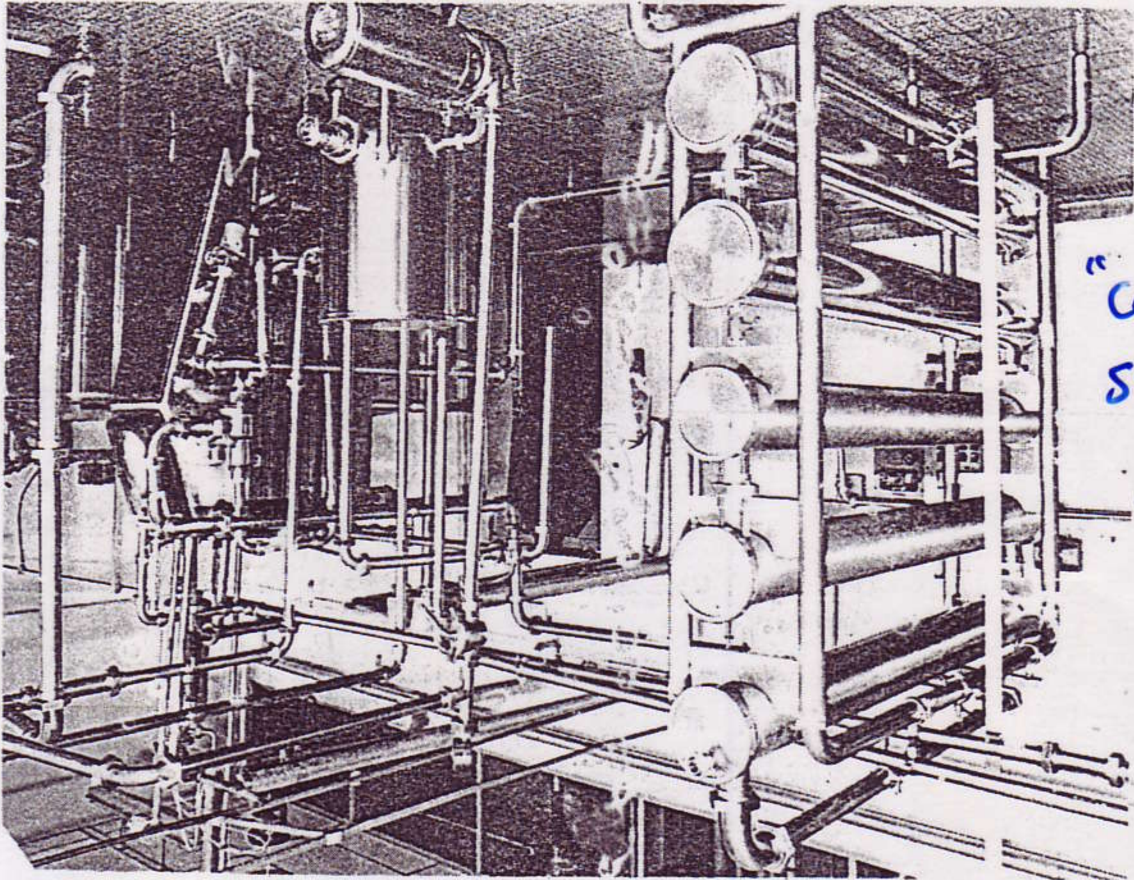
"BATCH STERILIZER" (DAIRY)





DAIRY : CONTINUOUS STERILIZATION (HEAT EXCHANGERS)



"CONTINUOUS"
STERILIZATION
PLANT

Steam Retort - cans

Continuous sterilisation plant (heat exchangers) – liquid foods

Effect of heat treatment of Polysaccharides

There have been only a few studies on this, and what data we have suggests that the damage depends very much on the type of polysaccharide. Consider 3 studies:

- **Bradley and Mitchell (1988) – alginate, carboxymethyl cellulose and κ-carrageenan**
- **Morris et al (1999) - low methoxy pectins**
- **Morris et al (2002) – high methoxy pectins**

Effect of heat treatment of Polysaccharides

1. Study by Bradley and Mitchell (1988)

Carbohydrate Polymers **9** (1988) 257–267

The Determination of the Kinetics of Polysaccharide Thermal Degradation using High Temperature Viscosity Measurements

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(Received 24 March 1988; accepted 14 April 1988)

ABSTRACT

Information about the thermal degradation of the polysaccharides sodium alginate, carrageenan and carboxymethyl cellulose has been obtained from the time dependence of the viscosity at high temperatures measured using a slit viscometer. The viscosity is related to the molecular weight using previously-published relations between the zero shear specific viscosity and the coil overlap parameter in conjunction with the appropriate Mark–Houwink equation. It is found that alginate is much less stable than carboxymethyl cellulose and carrageenan. Activation energies for depolymerisation obtained from Arrhenius plots in the presence of oxygen ranged from 50 kJ/mol for alginate to 105 kJ/mol for κ-carrageenan.

- used specially designed “slit viscometer” for measurement at high temperature (up to 100°C)
- Alginate, carboxymethyl cellulose, κ-carrageenan
- Measured change of specific viscosity η_{sp} with time at different temps. T
- Use Mark-Houwink relation $[\eta] = K'M^{a}$ to obtain molecular weight information
- Alginate much less stable

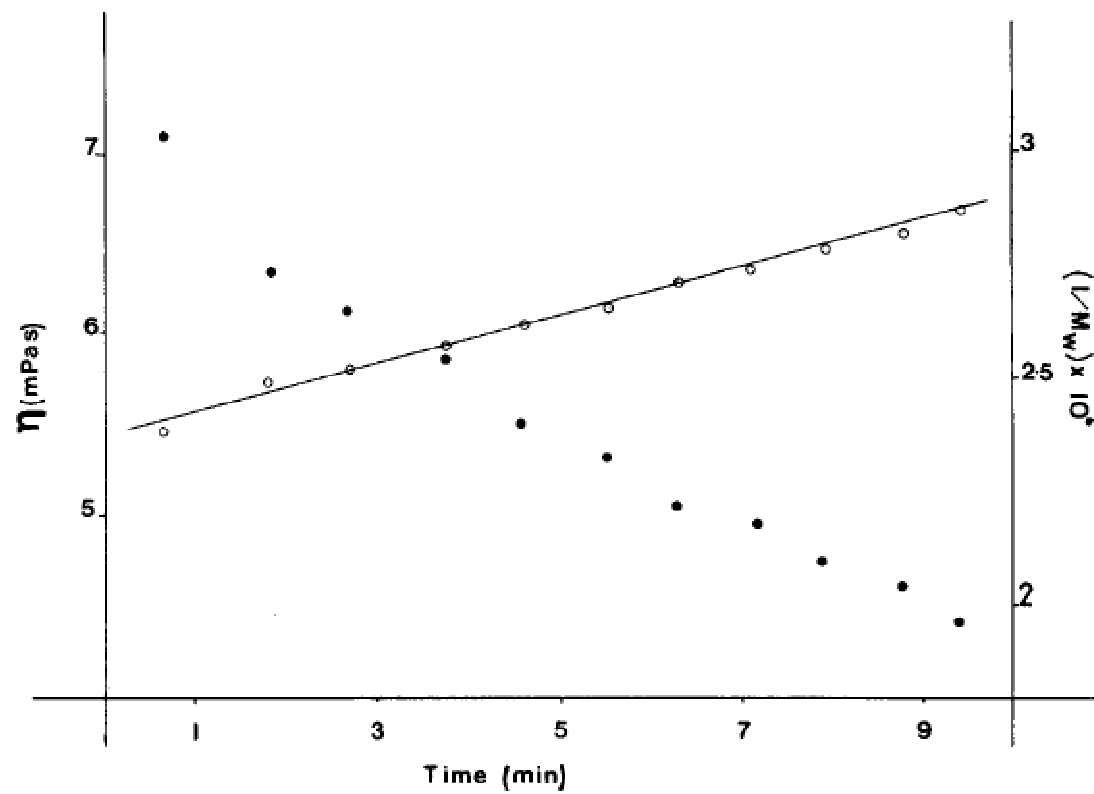

Fig. 2. Viscosity and reciprocal molecular weight of 1·5% κ-carrageenan at 118°C plotted against time. ●, Viscosity data; ○, $\bar{M}_w$ data.

Effect of heat treatment of Polysaccharides

2. Study by Morris et al (1999)

Progr Colloid Polym Sci (1999) 113:205–208
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BIOLOGICAL SYSTEMS

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Elevated-temperature analytical ultracentrifugation of a low-methoxy polyuronide

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Abstract Relatively little has been published on the ultracentrifuge behaviour of macromolecular solutions at elevated temperature (> 40 °C). In this study we look at the sedimentation velocity behaviour of one particular food grade polyuronide – pectin – from 20 °C to 60 °C in a specially adapted Model E ultracentrifuge. Reduced specific viscosity measurements were also determined over the same temperature range. A small decrease in the reduced viscosity, together with a similar increase in $s_{20,w}$, suggests that the pectin chain is more flexible at elevated temperatures, but that the overall molecular integrity remains intact.

Key words Beckman Model E adaptation · Viscometry · Size exclusion chromatography-multi-angle laser light scattering

- used specially designed analytical ultracentrifuge for measurement of sed. coeffs. and mol. wts. at temps up to 60°C – intrinsic viscosities measured too
- <u>Low methoxy pectins</u>
- No clear degradation - s, [η], M show little change with temp of measurement

No clear degradation - s, [η], M show little change with temp of measurement:

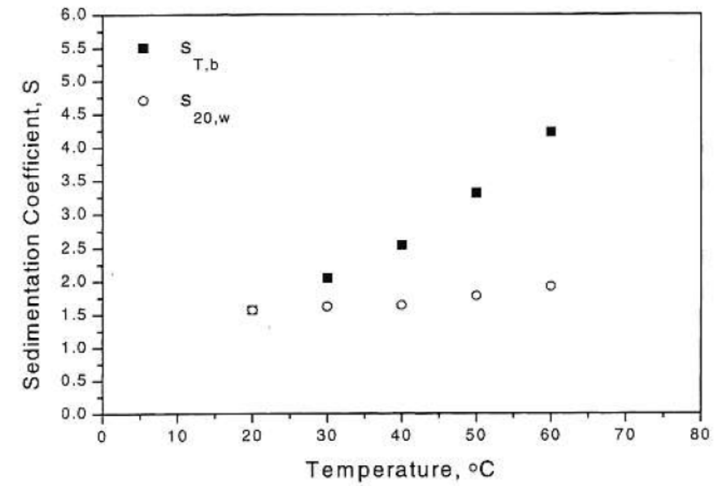


Fig. 3 The effect of increased temperature on s_{obs} and $s_{20,w}$ for LM pectin HL7192

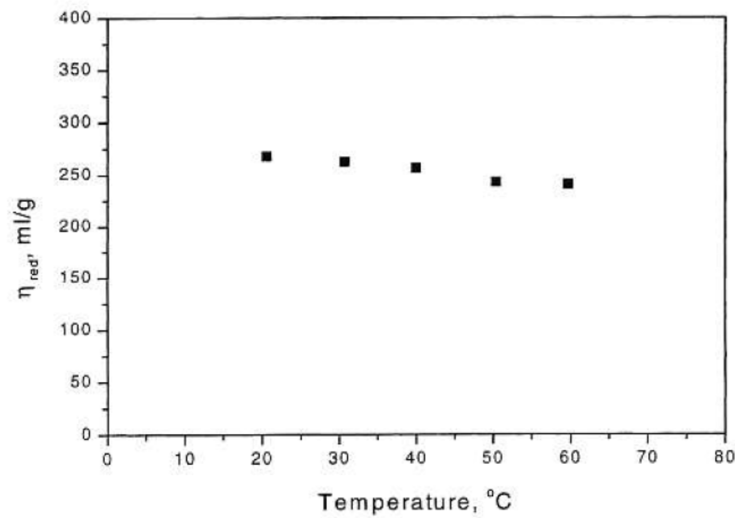


Fig. 4 The effect of increased temperature on the reduced viscosity for LM pectin HL7192

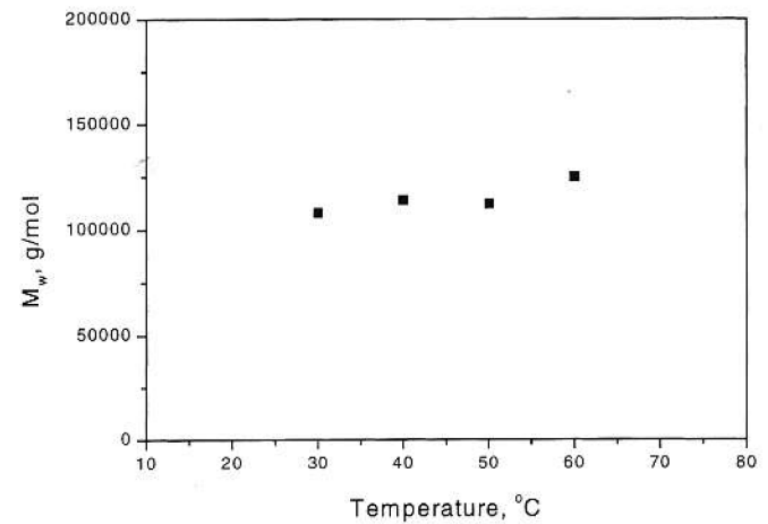
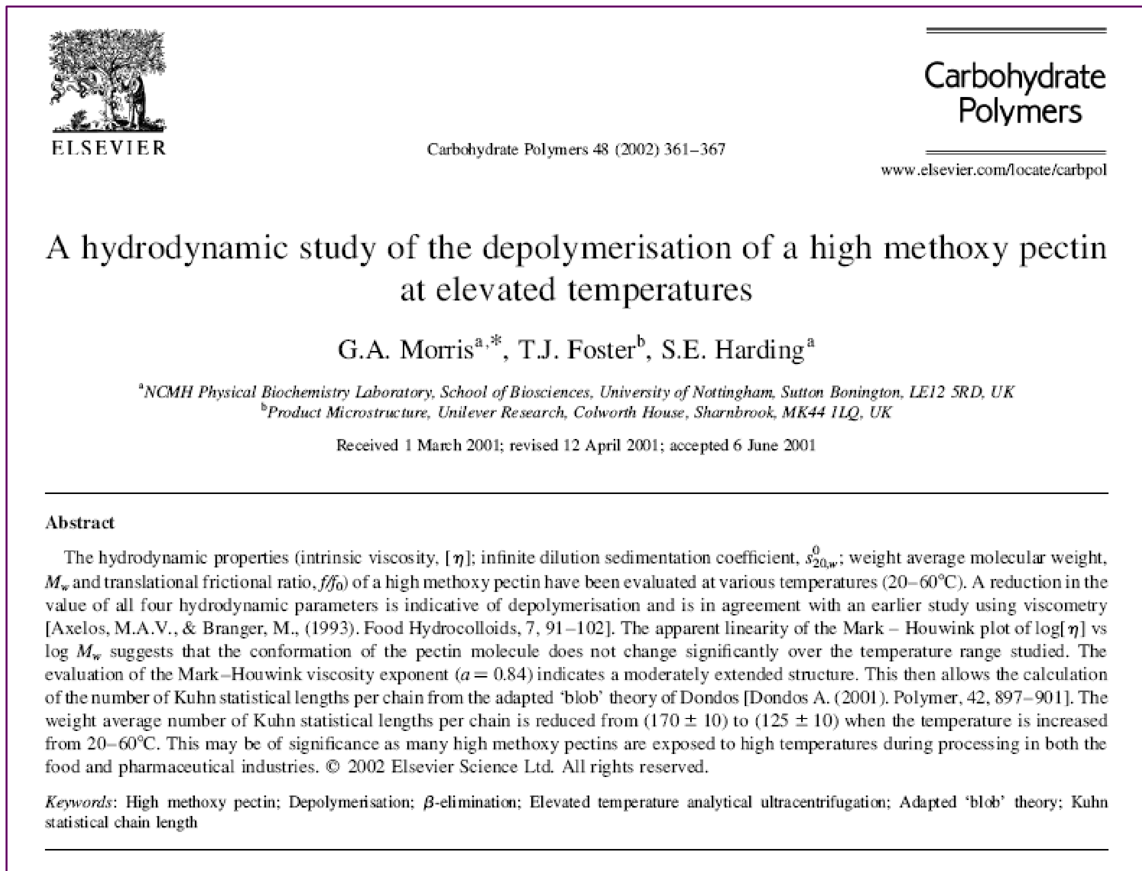


Fig. 5 The effect of increased temperature on the reduced viscosity for LM pectin HL7192

Effect of heat treatment of Polysaccharides

3. Study by Morris et al (2002)



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Carbohydrate Polymers 48 (2002) 361–367

Carbohydrate Polymers

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A hydrodynamic study of the depolymerisation of a high methoxy pectin at elevated temperatures

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Received 1 March 2001; revised 12 April 2001; accepted 6 June 2001

Abstract

The hydrodynamic properties (intrinsic viscosity, [η]; infinite dilution sedimentation coefficient, $s^0_{20,w}$; weight average molecular weight, M_w and translational frictional ratio, f/f_0) of a high methoxy pectin have been evaluated at various temperatures (20–60°C). A reduction in the value of all four hydrodynamic parameters is indicative of depolymerisation and is in agreement with an earlier study using viscometry [Axelos, M.A.V., & Branger, M., (1993). Food Hydrocolloids, 7, 91–102]. The apparent linearity of the Mark – Houwink plot of log[η] vs log M_w suggests that the conformation of the pectin molecule does not change significantly over the temperature range studied. The evaluation of the Mark–Houwink viscosity exponent ($a = 0.84$) indicates a moderately extended structure. This then allows the calculation of the number of Kuhn statistical lengths per chain from the adapted 'blob' theory of Dondos [Dondos A. (2001). Polymer, 42, 897–901]. The weight average number of Kuhn statistical lengths per chain is reduced from (170 ± 10) to (125 ± 10) when the temperature is increased from 20–60°C. This may be of significance as many high methoxy pectins are exposed to high temperatures during processing in both the food and pharmaceutical industries. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: High methoxy pectin; Depolymerisation; β-elimination; Elevated temperature analytical ultracentrifugation; Adapted 'blob' theory; Kuhn statistical chain length

- **High methoxy pectins**
- **In contrast to the result for LM pectins, clear degradation - s, [η], M all decrease with temp of measurement, although conformation (from $[\eta] = K'M^a$ and $s = K''M^b$ analyses) ~ unaltered**
- **Thermal stability of pectins seems to depend strongly on degree of esterification**

Effect of temperature (of measurement) on a high methoxy pectin

Temperature (°C)	[η] (ml/g)	$s^{0}_{20,w}$ (S)	M_w (g/mol)	f/f_0
20	406 ± 2	1.83 ± 0.01	156,000 ± 10,000	8.2 ± 0.4
30	387 ± 4	1.81 ± 0.02	144,500 ± 10,000	7.9 ± 0.3
40	362 ± 4	1.79 ± 0.01	133,000 ± 10,000	7.5 ± 0.3
50	338 ± 3	1.77 ± 0.02	126,500 ± 10,000	7.4 ± 0.5
60	321 ± 8	1.76 ± 0.01	116,700 ± 10,000	6.9 ± 0.4

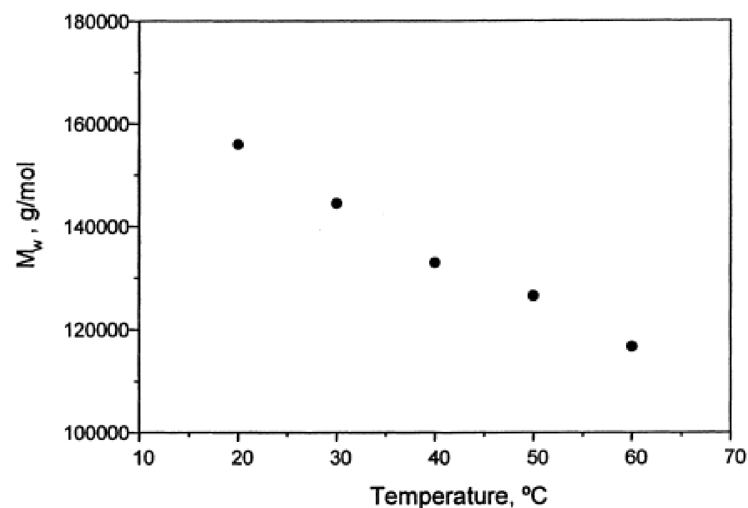

Fig. 6. Effect of increased temperature on the weight average molecular weight, M_w for a high-methoxy pectin in standard phosphate chloride buffer (pH = 6.8, I = 0.1 M).

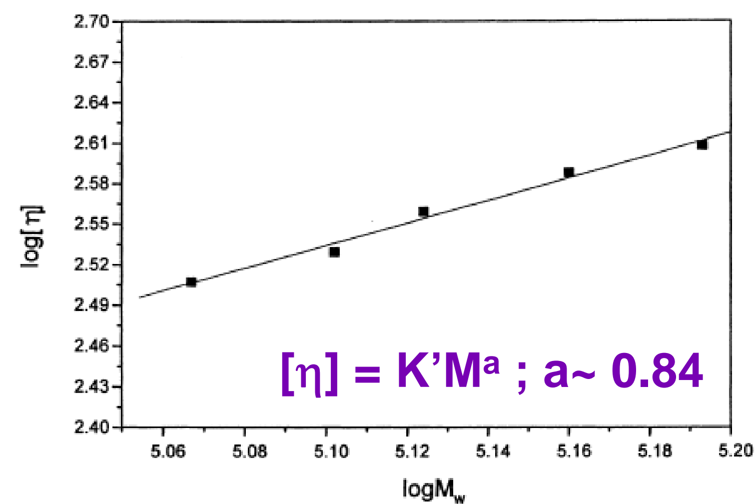

Fig. 8. Mark–Houwink (MHKS) plot for a high-methoxy pectin at different temperatures.

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