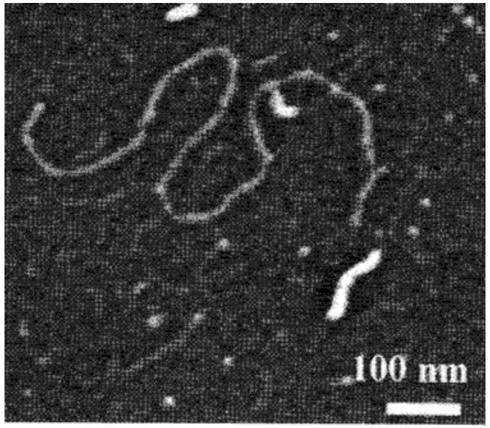
The physical characterisation of polysaccharides in solution



Stephen Harding

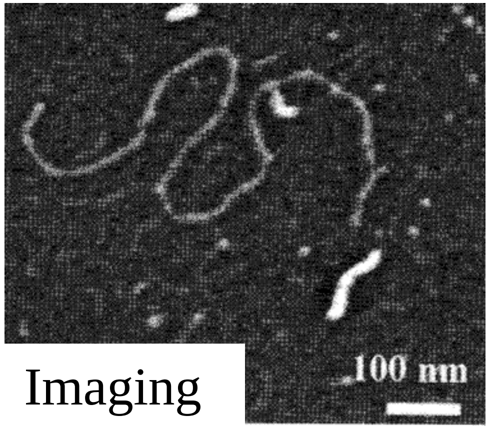
University of Nottingham

The physical characterisation of polysaccharides in solution


Viscometry


SEC-MALLs

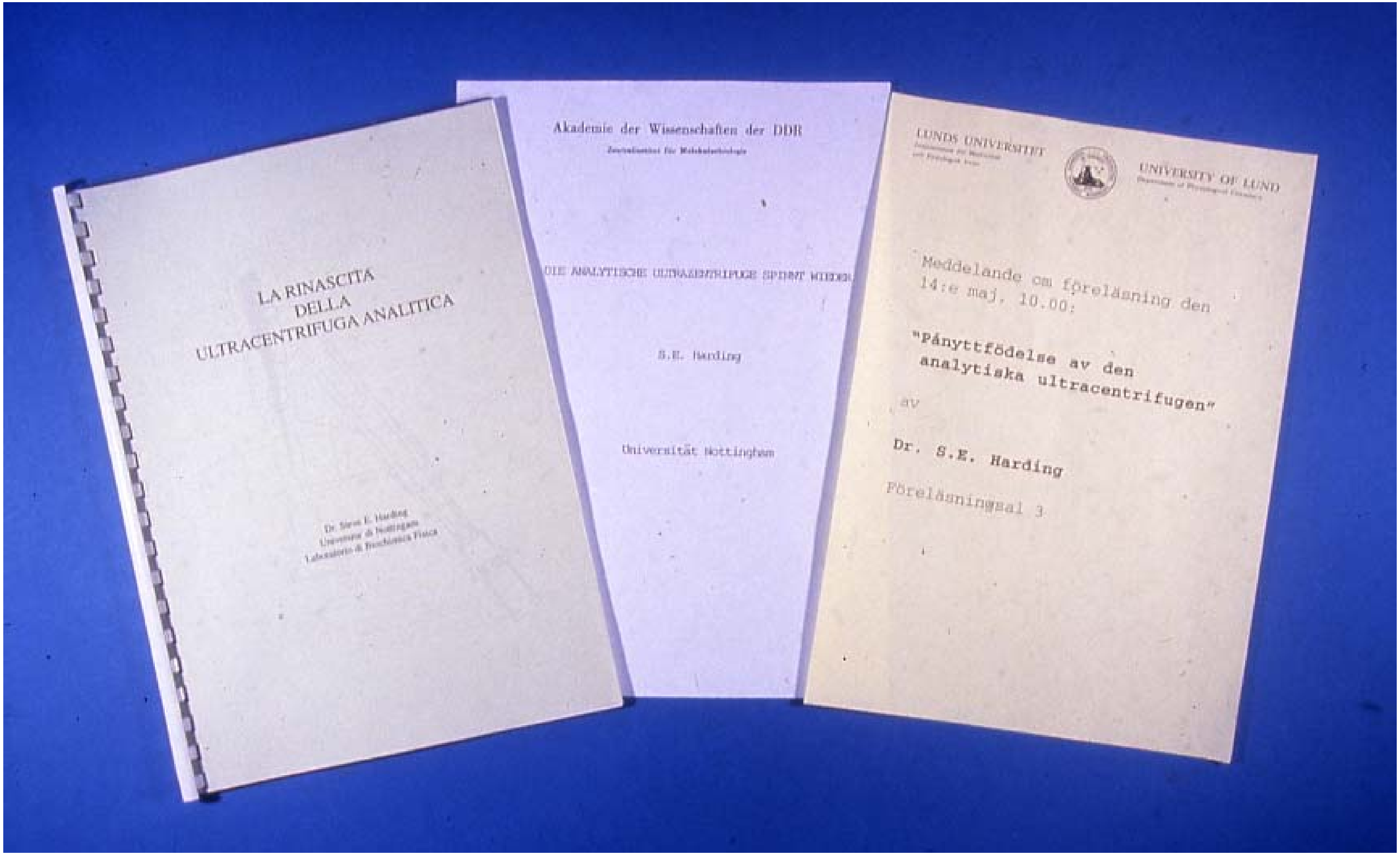

Analytical Ultracentrifugation

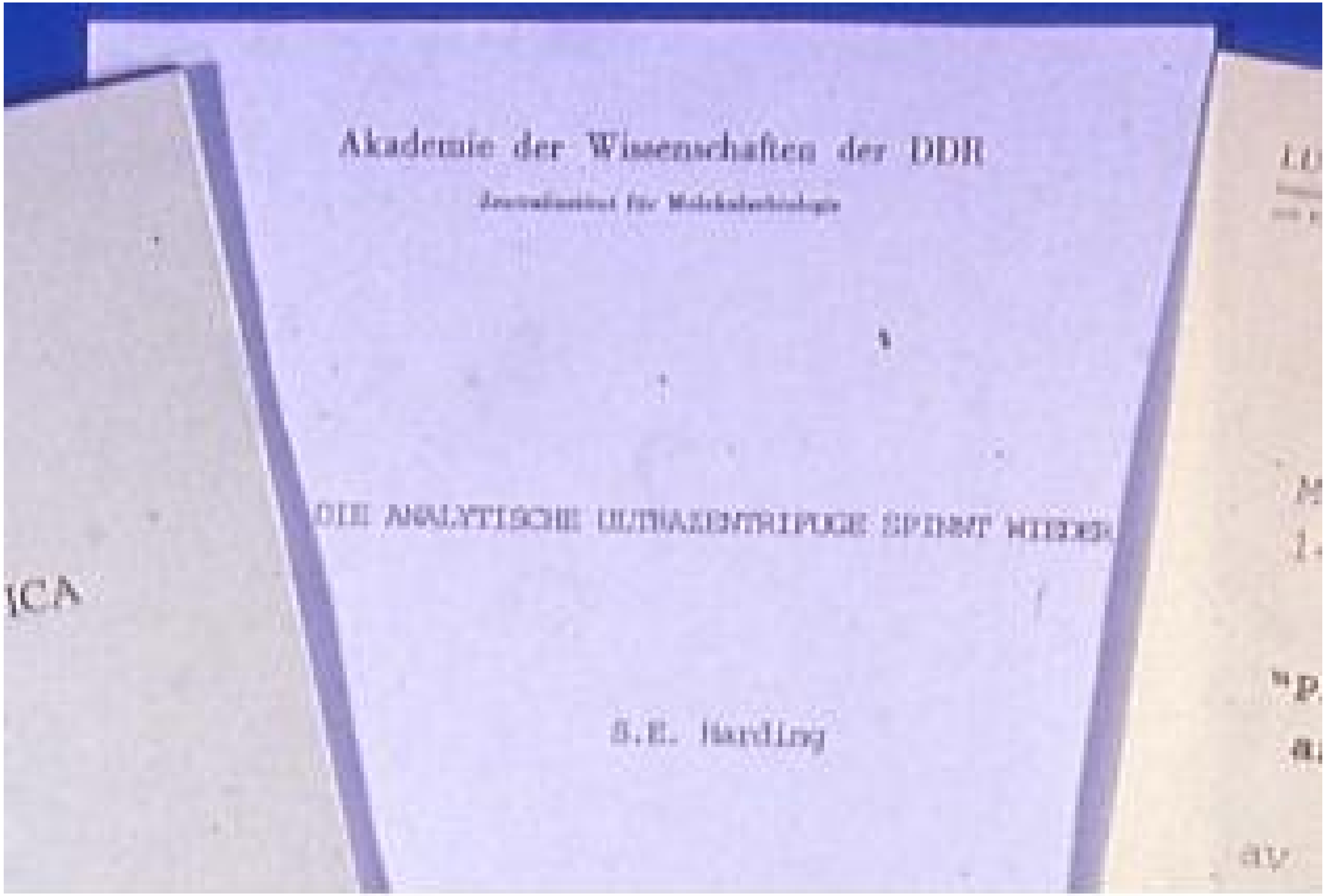


Imaging

Stephen Harding

University of Nottingham


LA RINASCITA
DELLA
ULTRACENTRIFUGA ANALITICA
Akademie der Wissenschaften der DDR
DIE ANALYTISCHE ULTRAZENTRIFUGE SPINNT WIEDER
S.E. Harding
Universität Nottingham
LUNDS UNIVERSITET
UNIVERSITY OF LUND
Meddelande om föreläsning den 14:e maj, 10.00:
"Pånyttfödelse av den analytiska ultracentrifugen"
av
Dr. S.E. Harding
Föreläsningsal 3


Akademie der Wissenschaften der DDR
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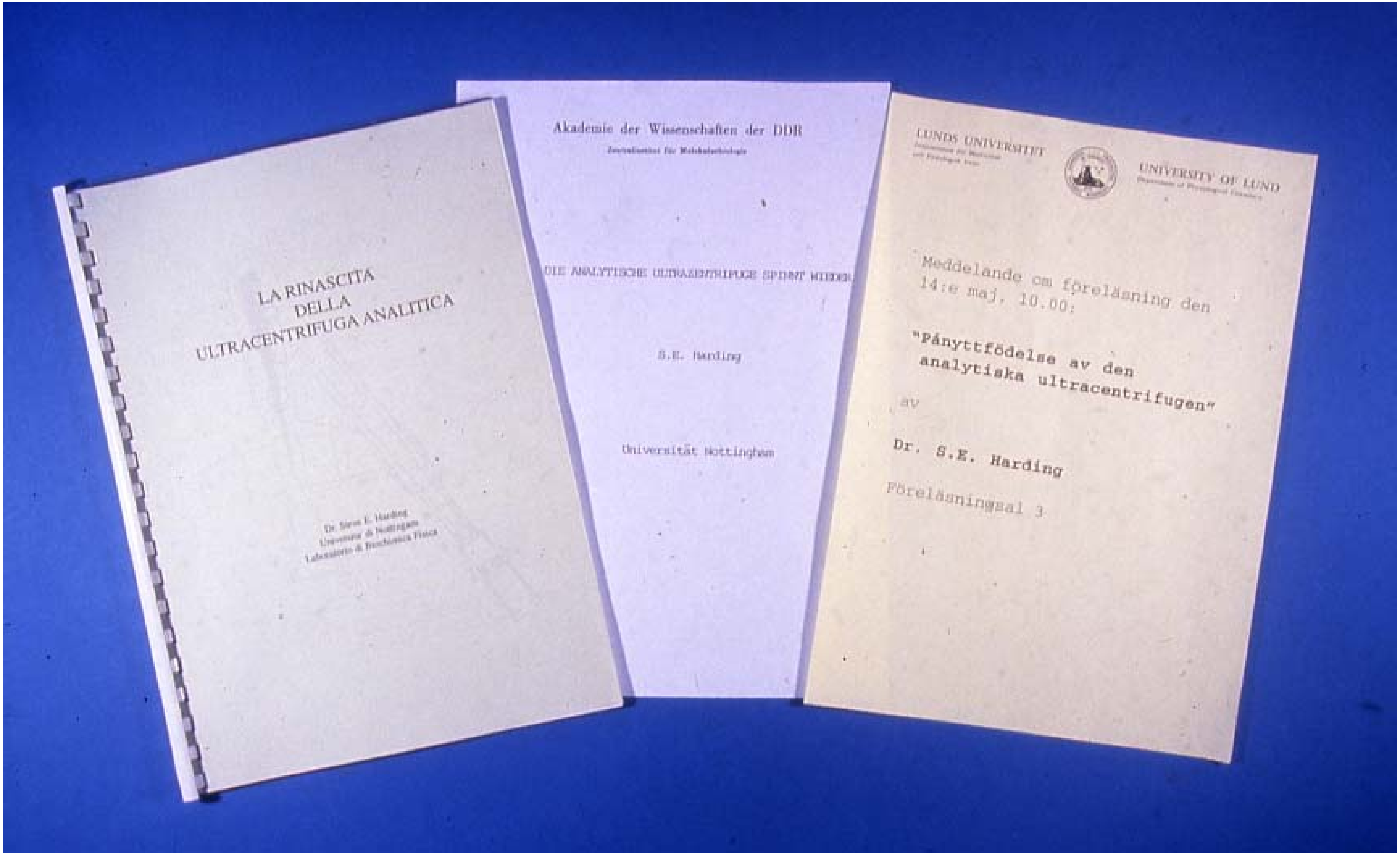
S.E. Harding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Physical characterisation

1. Viscosity, stability
2. Heterogeneity, Molecular weight & distribution, stability
3. Conformation in solution
4. Interactions

Physical characterisation

1. Viscosity, stability
2. Heterogeneity, Molecular weight & distribution
3. Conformation in solution
4. Interactions

1: Viscometry. 2: SEC-MALLs & analytical ultracentrifugation. 3: Viscometry, SEC-MALLs & analytical ultracentrifugation. 4. Analytical ulttracentrifugation & atomic force microscopy

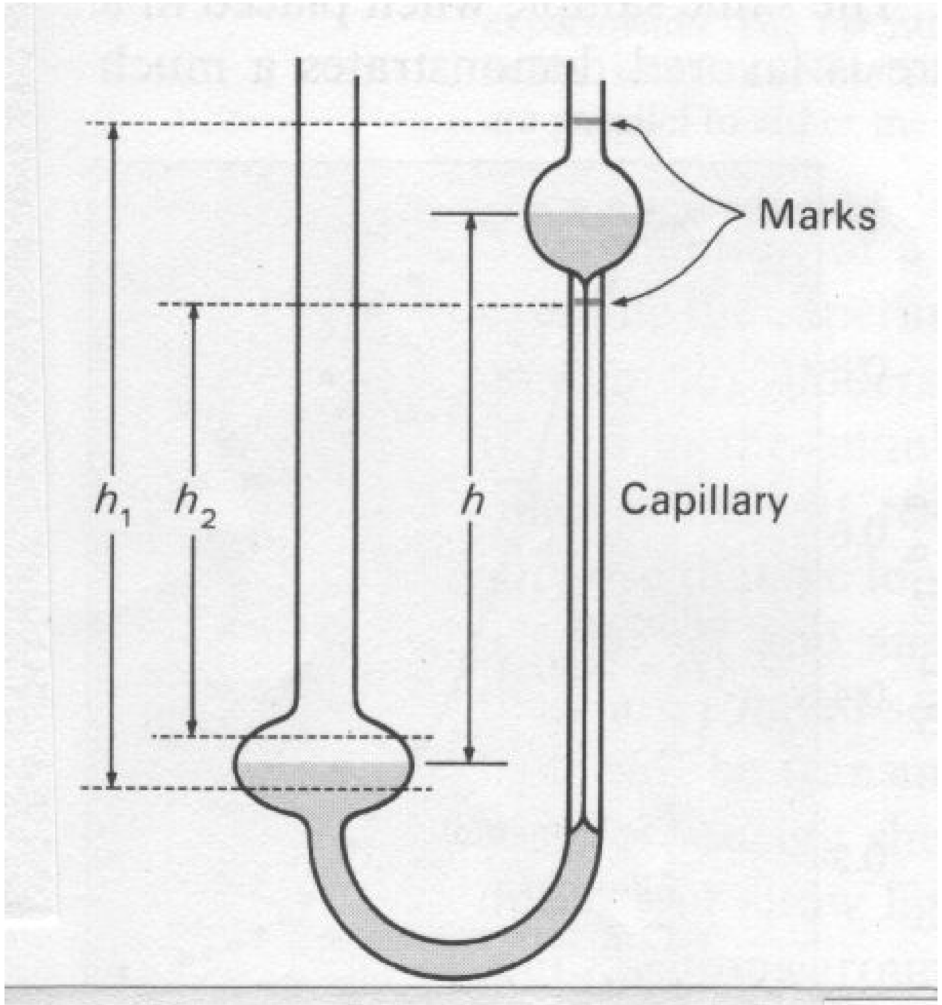
1. Viscosity from precision viscometry

1. Viscosity by Precision viscometry

$\Rightarrow$ $[\eta]$

Intrinsic viscosity, ml/g

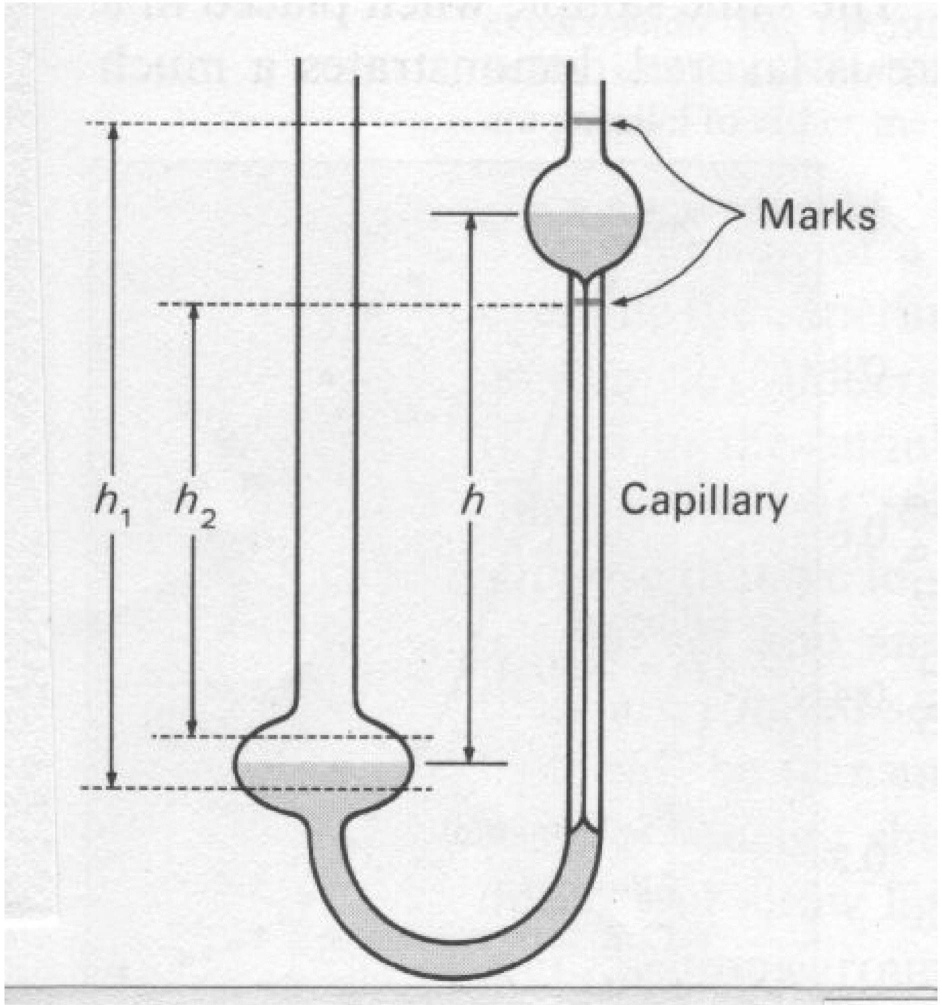
Types of Viscometer:



Ostwald Viscometer

Types of Viscometer:

1. "U-tube" (Ostwald or Ubbelohde)

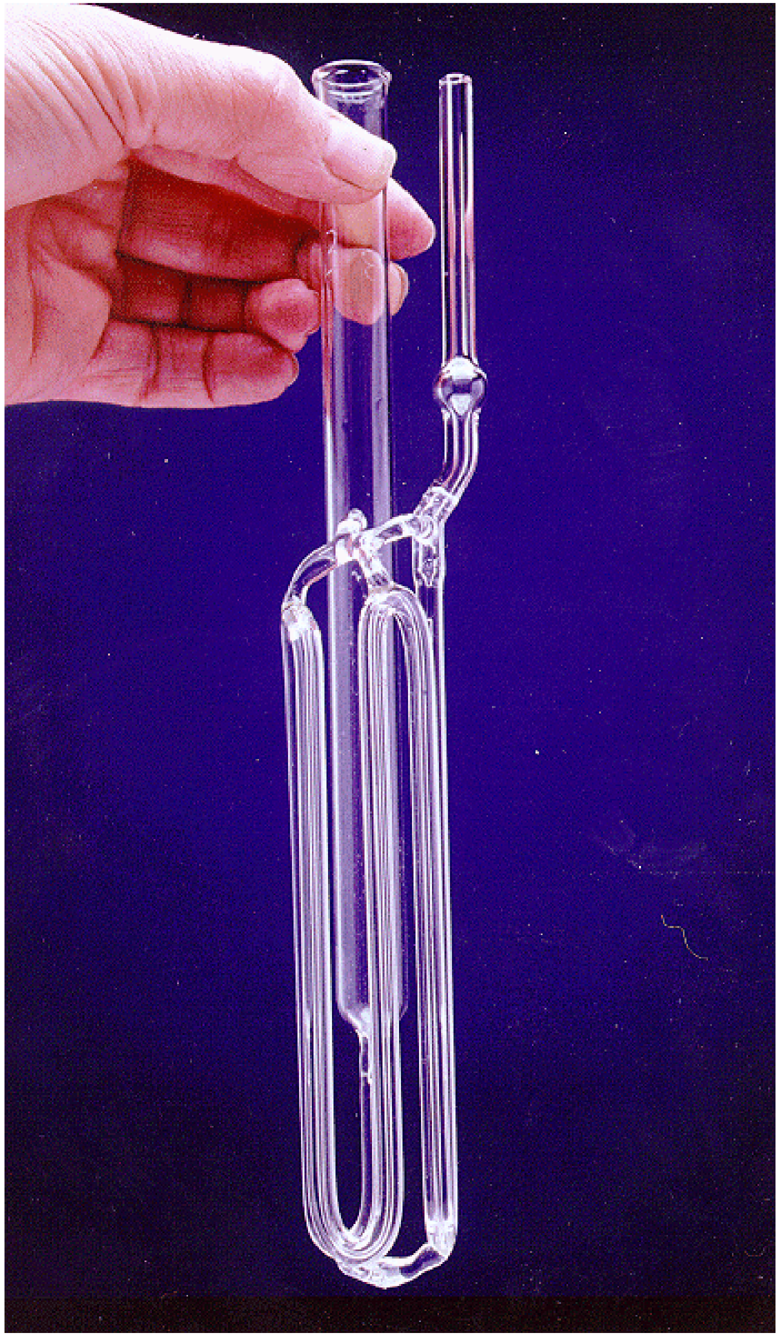


Ostwald Viscometer

Types of Viscometer:

1. "U-tube" (Ostwald or Ubbelohde)

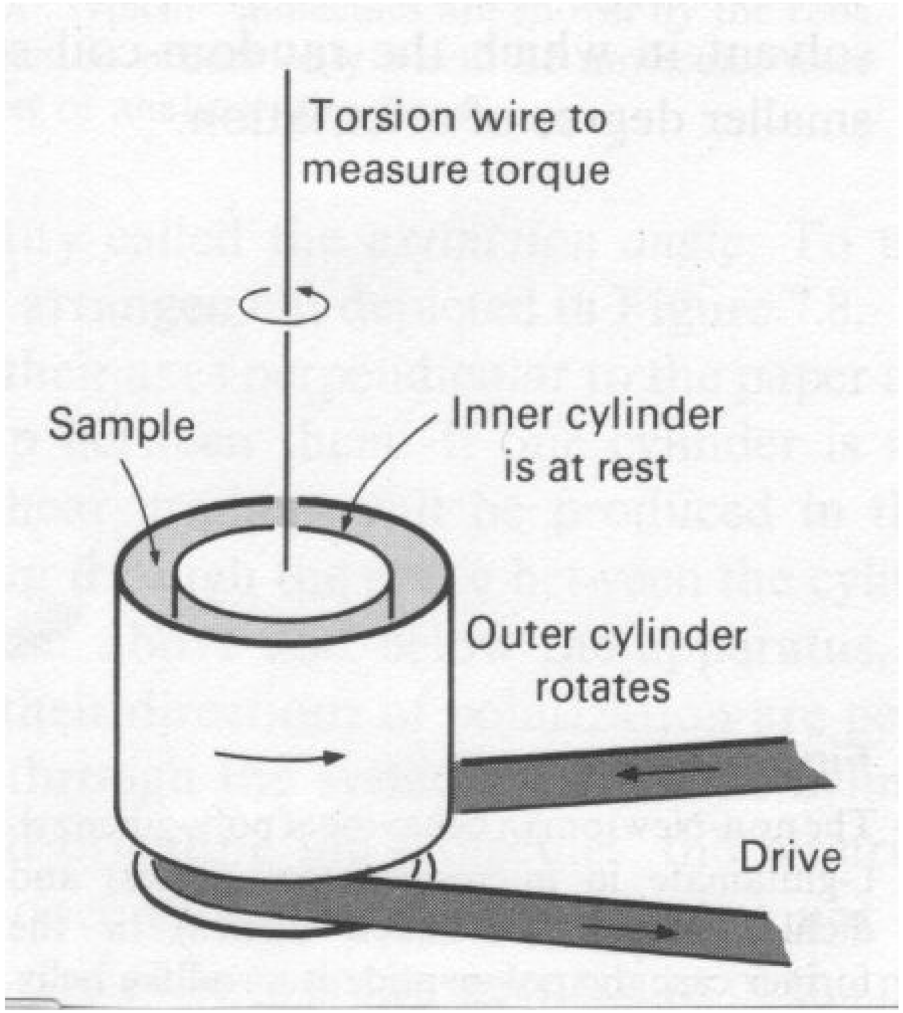
Extended Ostwald Viscometer



Types of Viscometer:

1. "U-tube" (Ostwald or Ubbelohde)

2. "Cone & Plate" (Couette)



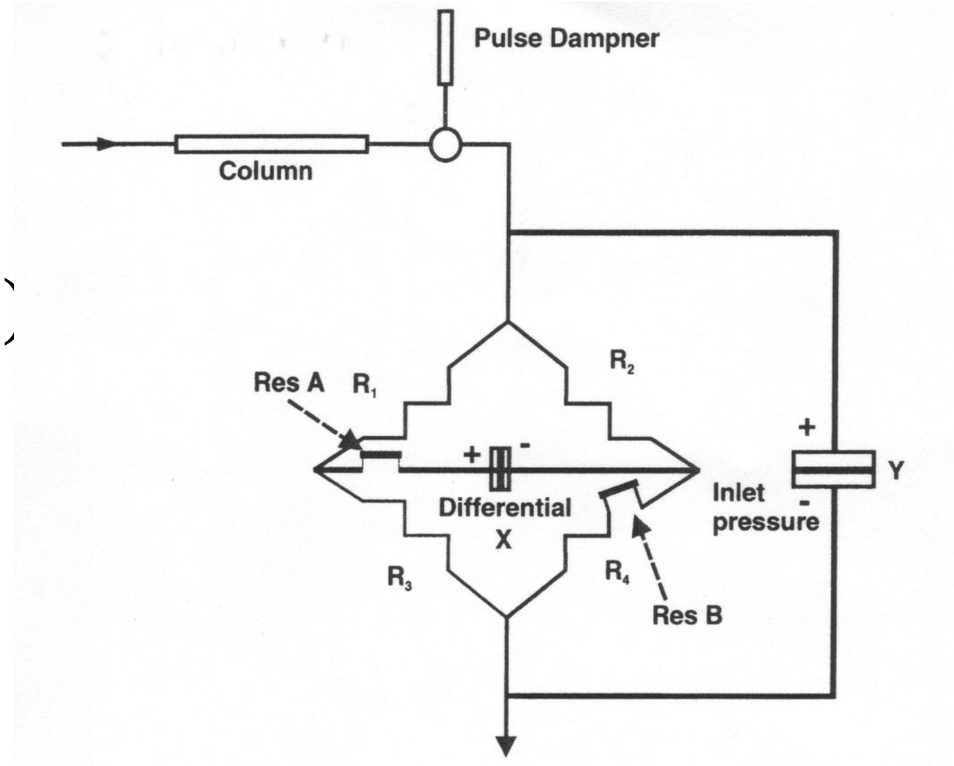
Couette-type Viscometer

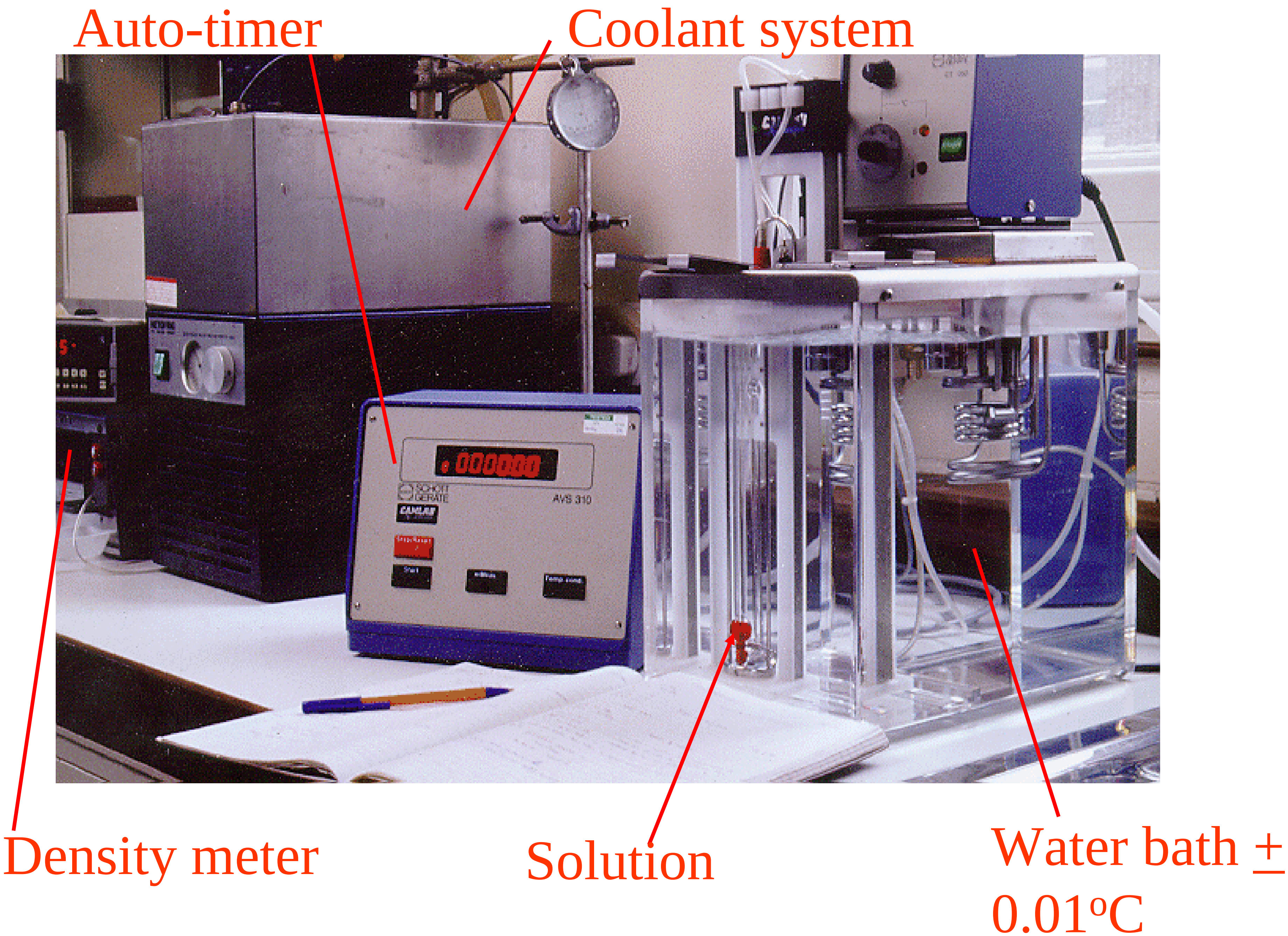
Types of Viscometer:

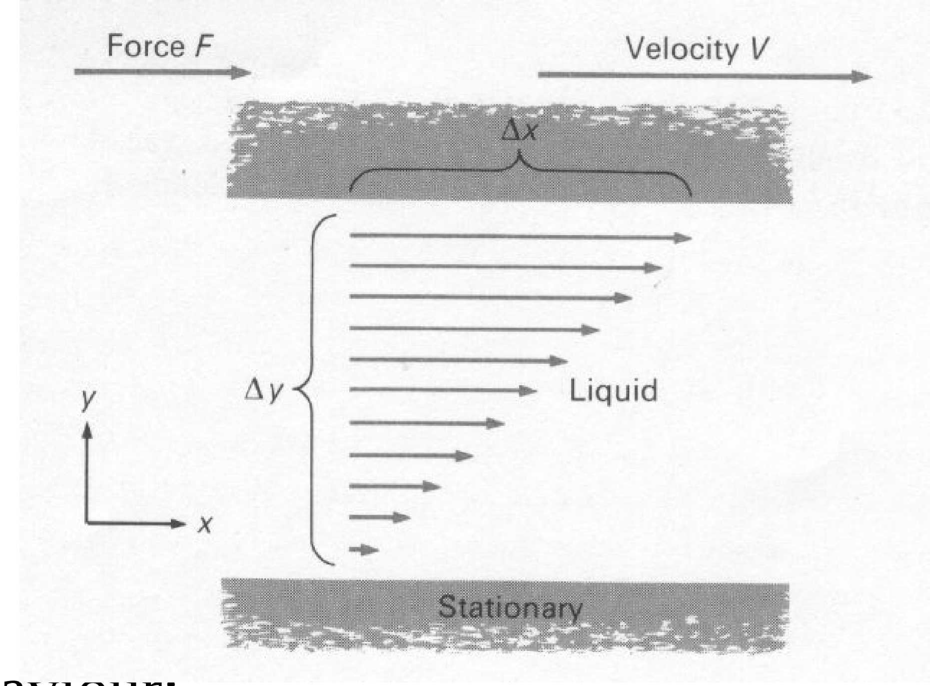
1. "U-tube" (Ostwald or Ubbelohde)

2. "Cone & Plate" (Couette)

3. Pressure imbalance on-line viscometer




Auto-timer
Coolant system
Density meter
Solution
Water bath ± 0.01°C
AVS 310



Definition of viscosity:

For normal (Newtonian) flow behaviour:

$\tau = (F/A) = \eta \cdot (dv/dy)$

viscosity

shear rate

shear stress

$$\eta = \tau/(dv/dy)$$

units: $(dyn/cm^2)/sec^{-1}$

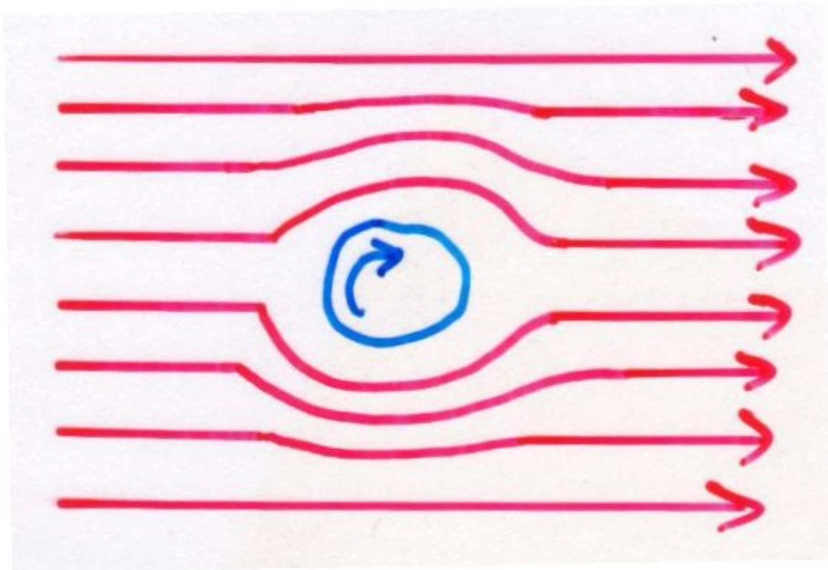
$= dyn.sec.cm^{-2}$.

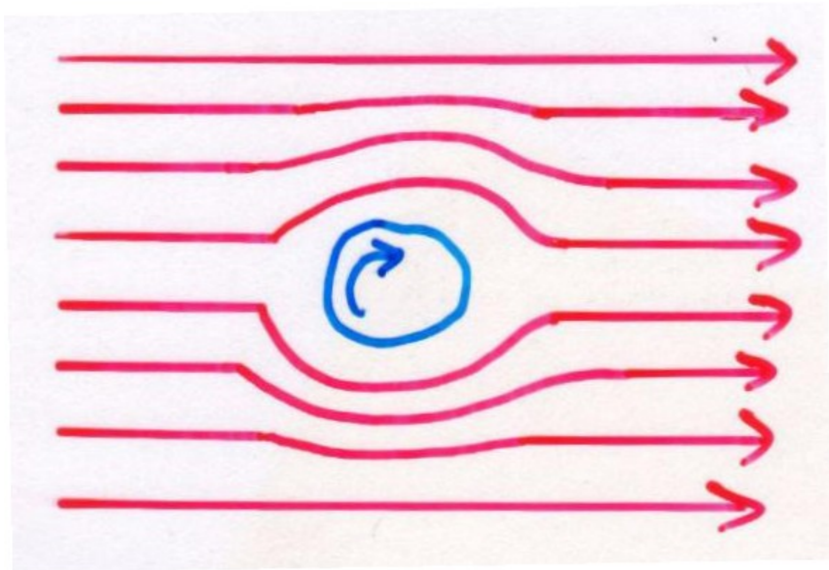
= POISE (P)

At 20.0°C, η(water) ~ 0.01P

Viscosity of biomolecular solutions:

A dissolved macromolecule will INCREASE the viscosity of a solution because it disrupts the streamlines of the flow:





We define the relative viscosity η_r as the ratio of the viscosity of the solution containing the macromolecule, η, to that of the pure solvent in the absence of macromolecule, η_o:

$$\eta_r = \eta/\eta_o$$ no units

For a U-tube viscometer, $\eta_r = (t/t_o).(\rho/\rho_o)$

Reduced viscosity

The relative viscosity depends (at a given temp.) on the concentration of macromolecule, the shape of the macromolecule & the volume it occupies.

If we are going to use viscosity to infer on the shape and volume of the macromolecule we need to eliminate the concentration contribution.

The first step is to define the reduced viscosity

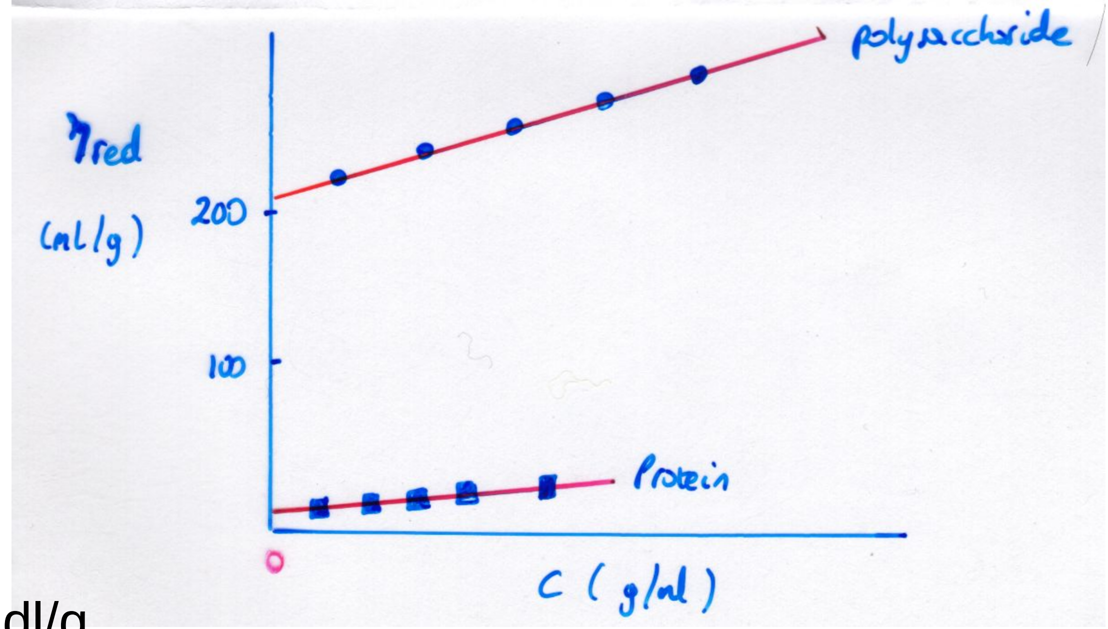
$$\eta_{red} = (\eta_r - 1)/c$$

If c is in g/ml, units of η_{red} are ml/g

The Intrinsic Viscosity [η]

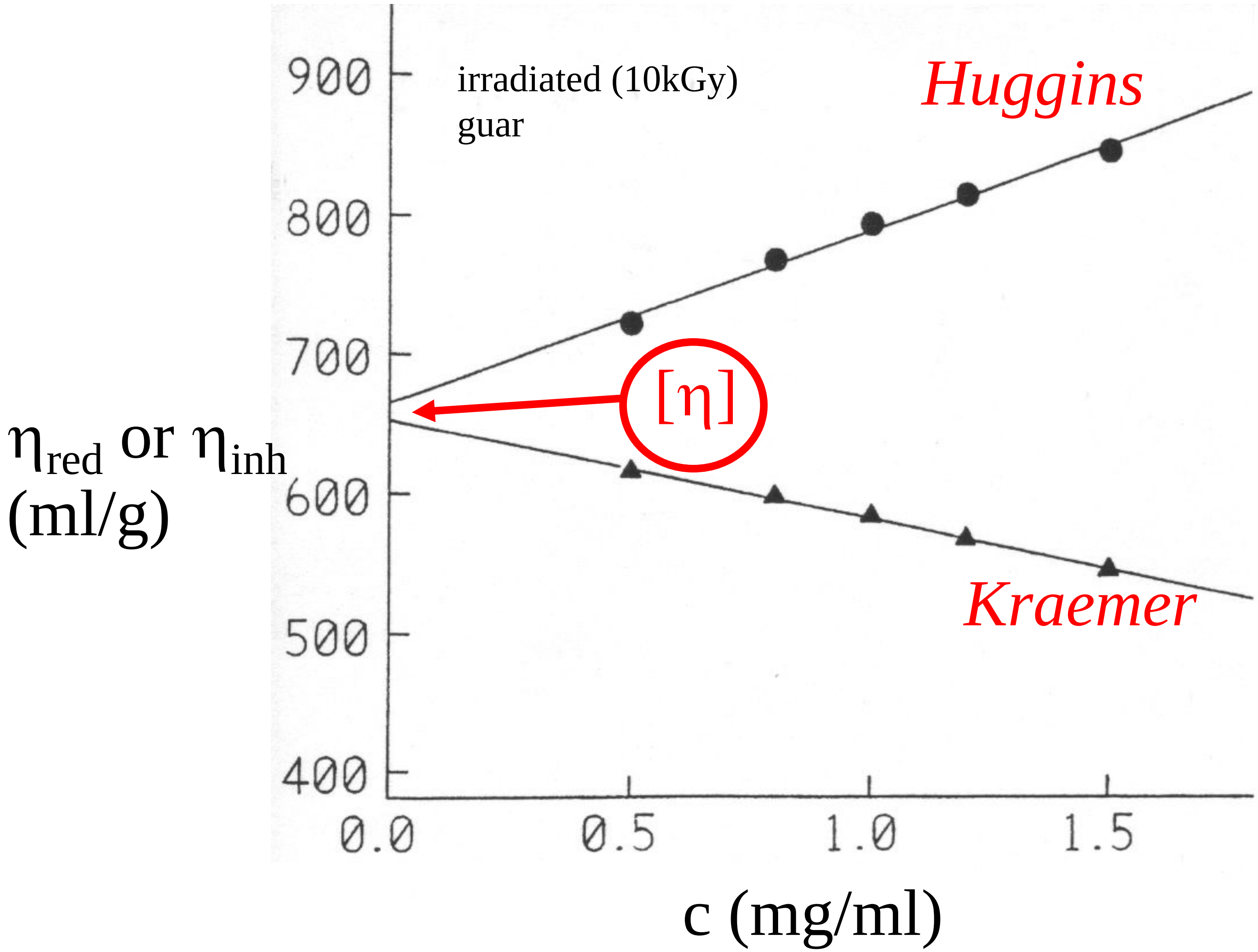
The next step is to eliminate non-ideality effects deriving from exclusion volume, backflow and charge effects. We measure η_{red} at a series of concentrations and extrapolate to zero concentration:

$$[\eta] = \mathrm{Lim}_{c \rightarrow 0} (\eta_{red})$$

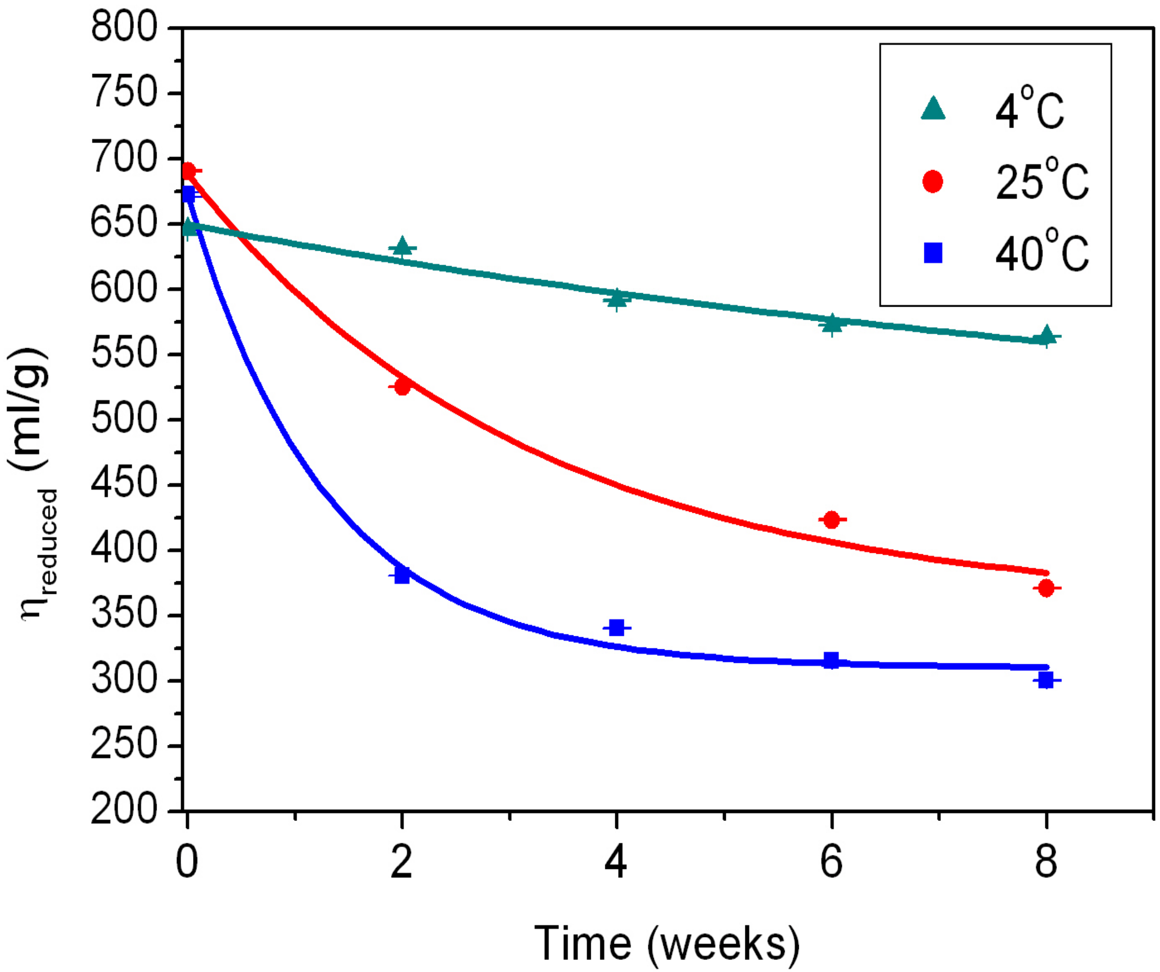


units [η] = ml/g

sometimes researchers use dl/g
so 200 ml/g = 2 dl/g


irradiated (10kGy)
guar
Huggins
Kraemer
[η]
900
800
700
600
500
400
0.0
0.5
1.0
1.5
ηred or ηinh
(ml/g)
c (mg/ml)

Viscosity probe: chitosan stability

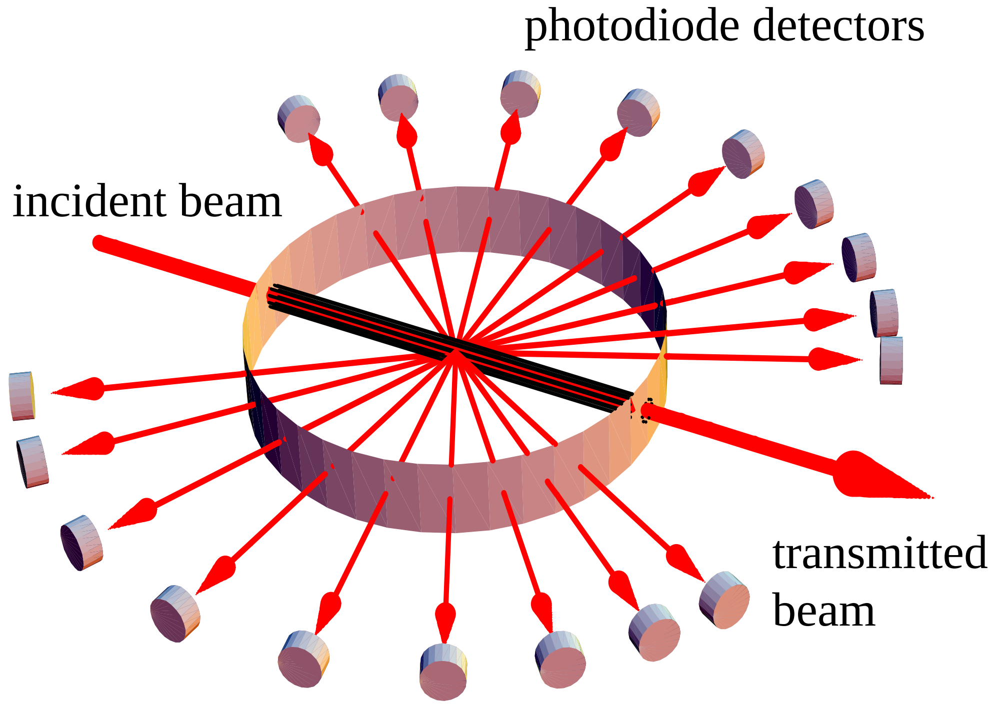


Fee et al, 2006

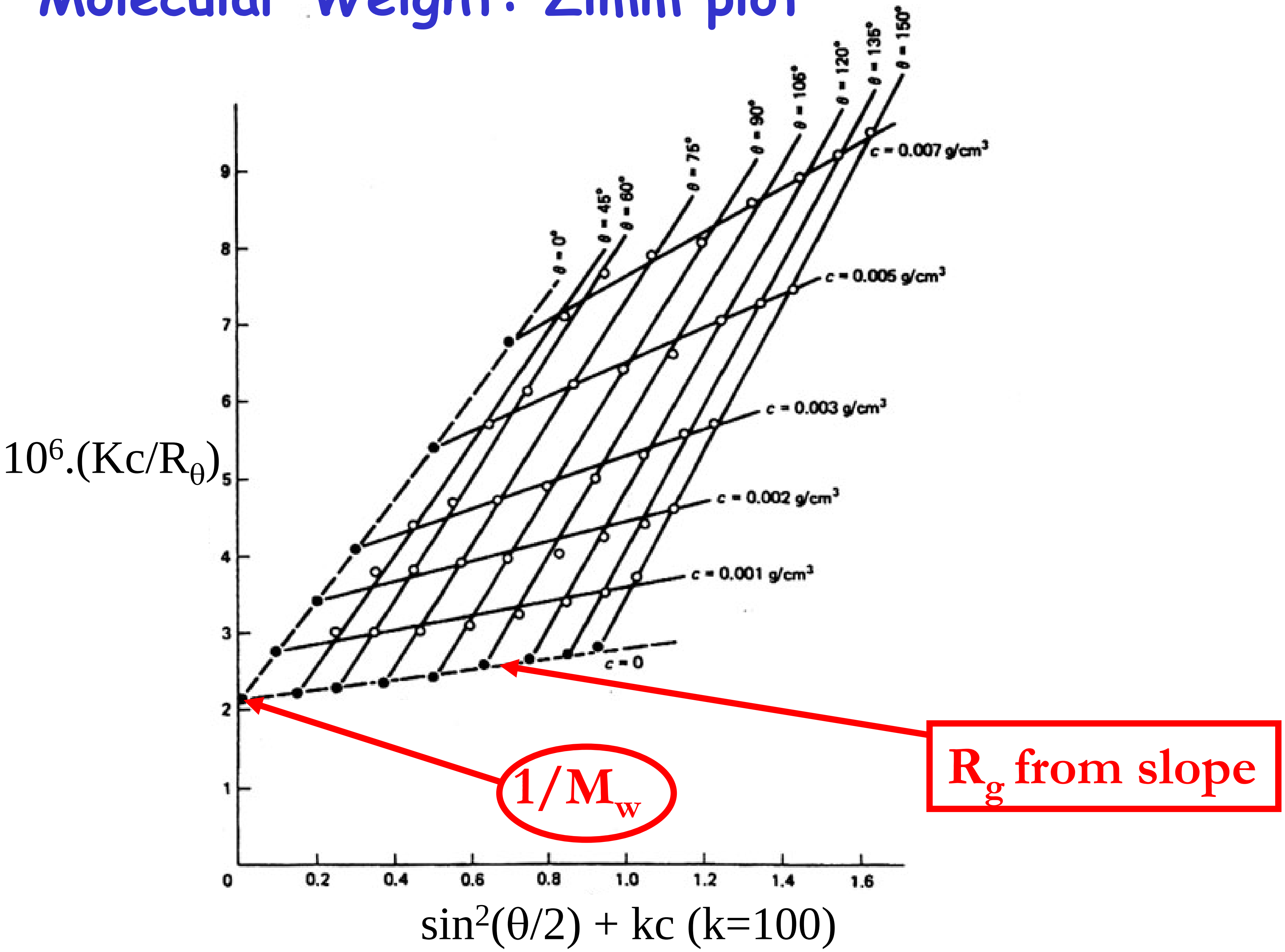
2. Heterogeneity and molecular weight: SEC-MALLs

2. Heterogeneity and Molecular Weight: SEC-MALLs

Molecular Weight: Light scattering

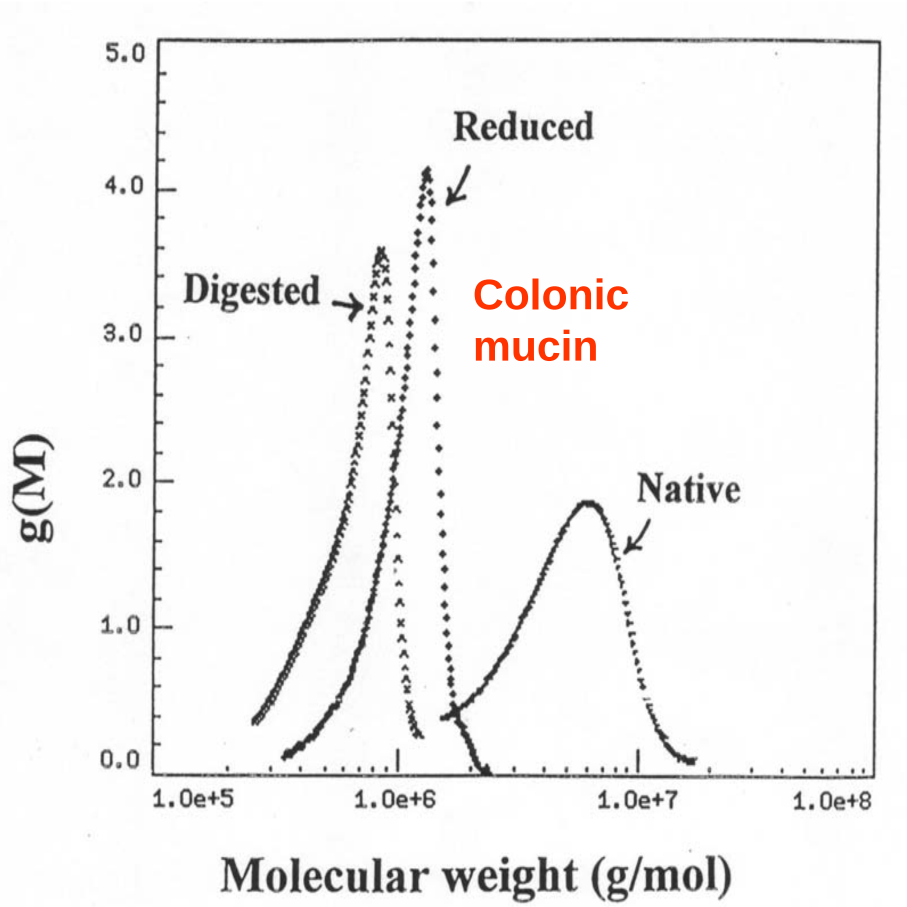


Molecular Weight: Zimm plot

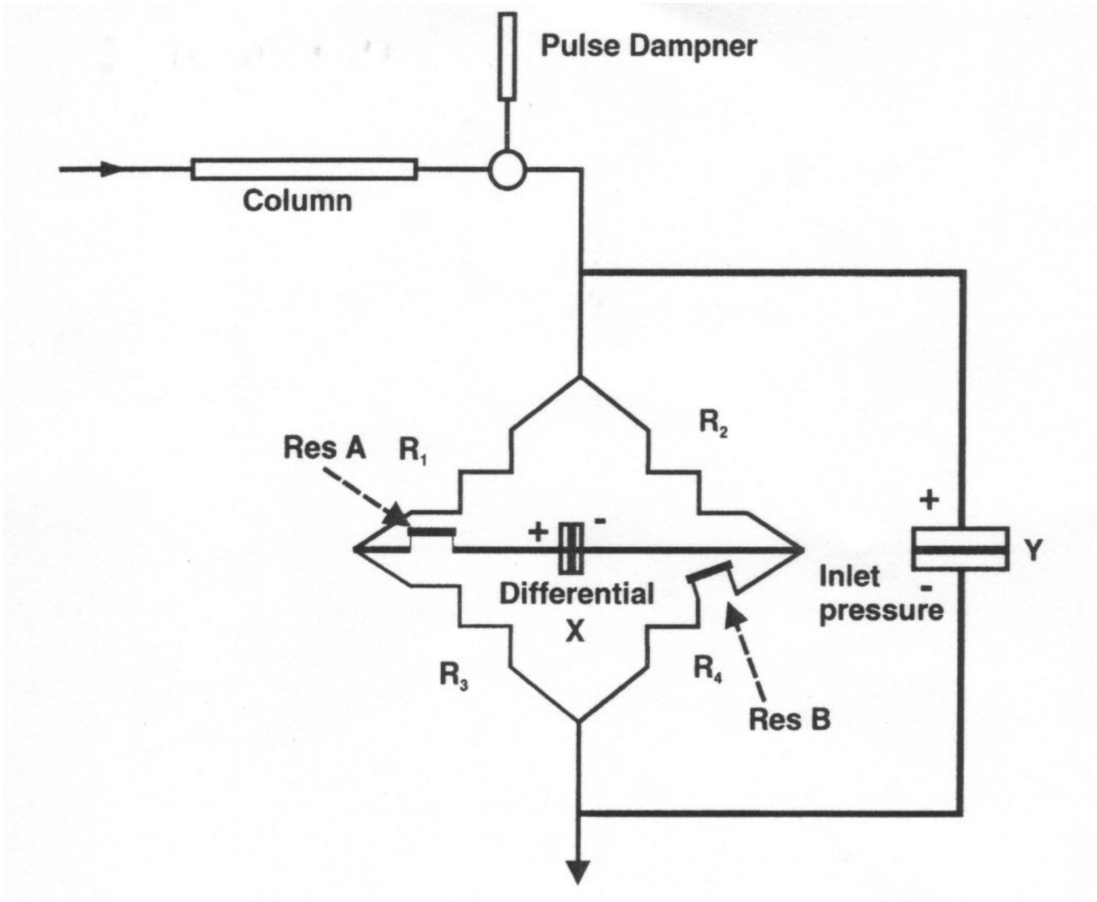


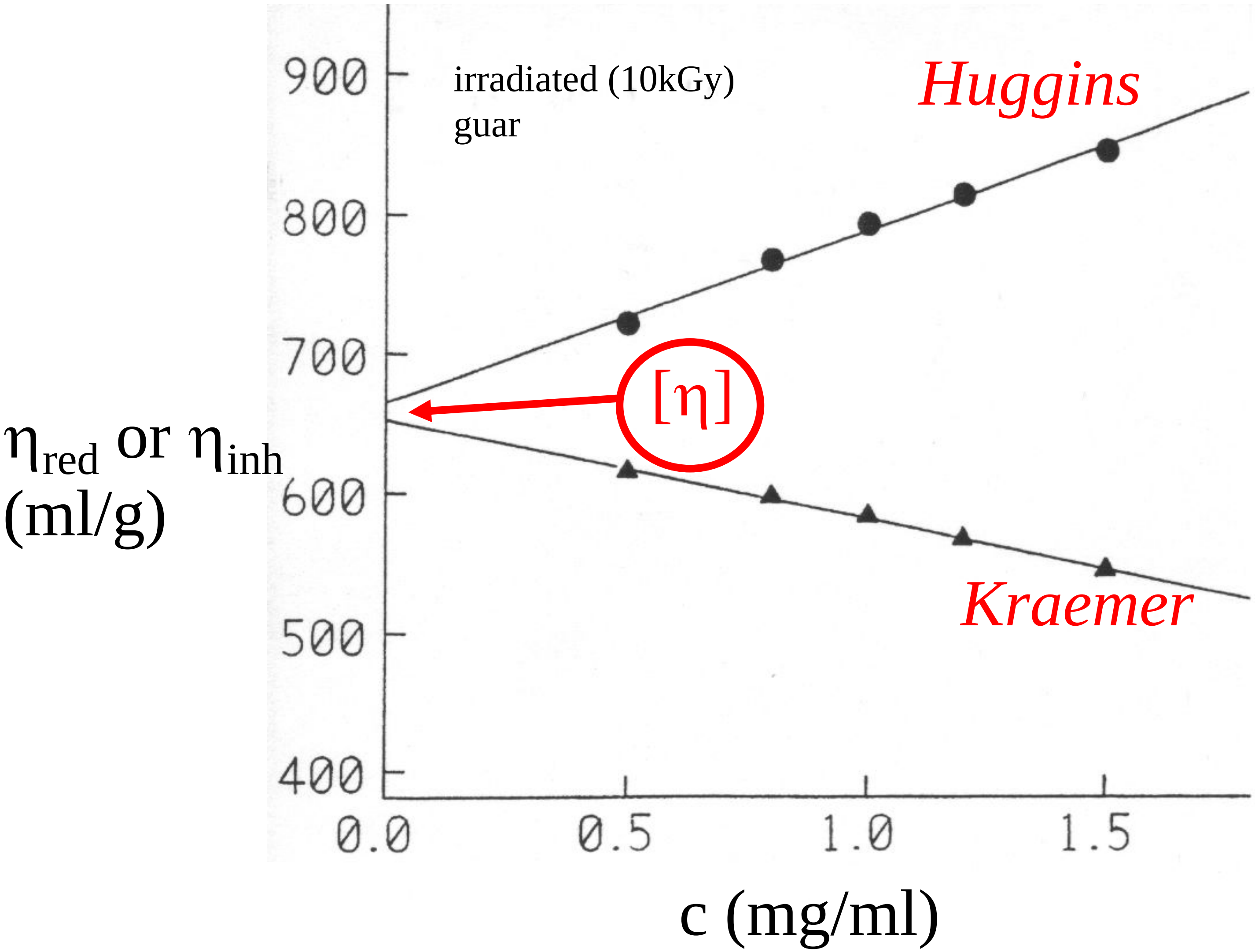
Molecular Weight: SEC-MALLS

Molecular Weight: SEC-MALLS

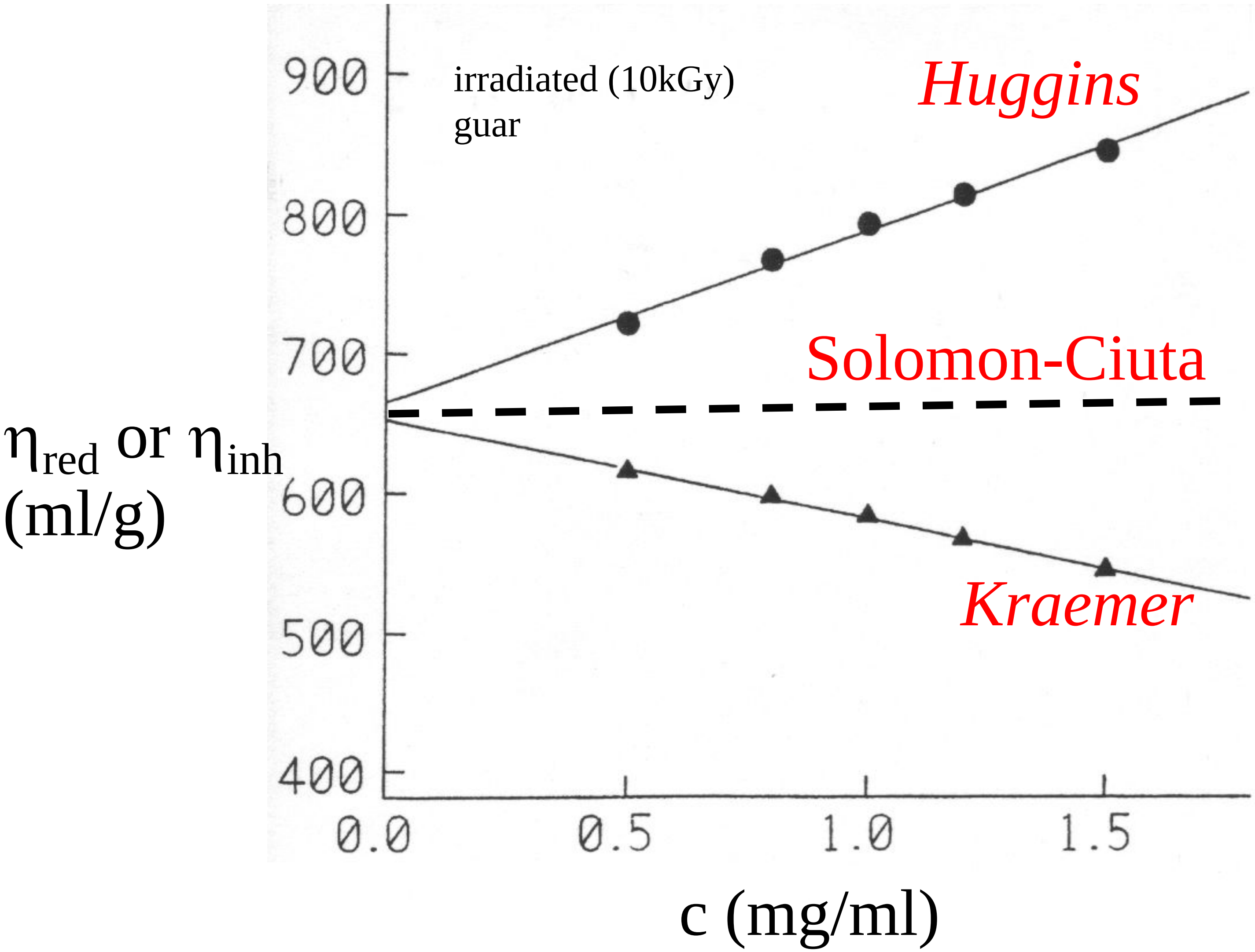


Can also couple a "pressure imbalance" type of viscometer on-line…





irradiated (10kGy)
guar
Huggins
Kraemer
[η]
900
800
700
600
500
400
0.0
0.5
1.0
1.5
η_{red} or η_{inh}
(ml/g)
c (mg/ml)



irradiated (10kGy)
guar
Huggins
Solomon-Ciuta
Kraemer
900
800
700
600
500
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ηred or ηinh
(ml/g)
0.0
0.5
1.0
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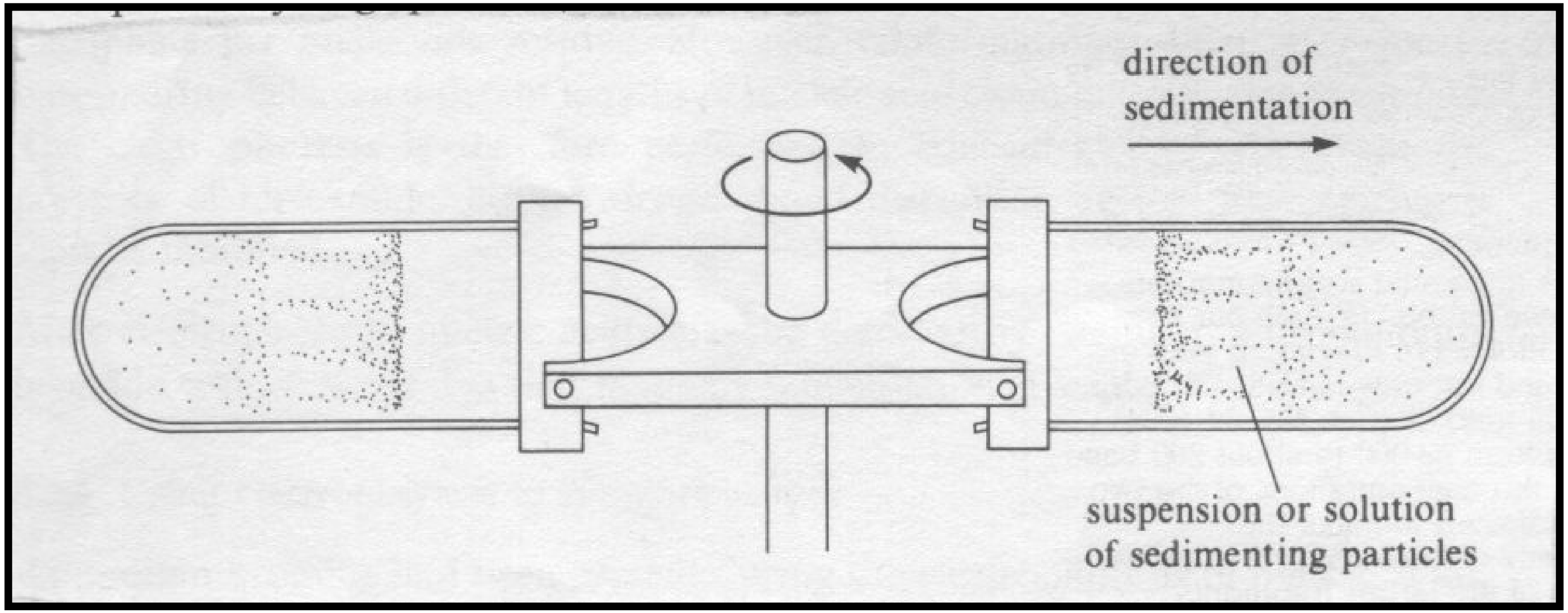
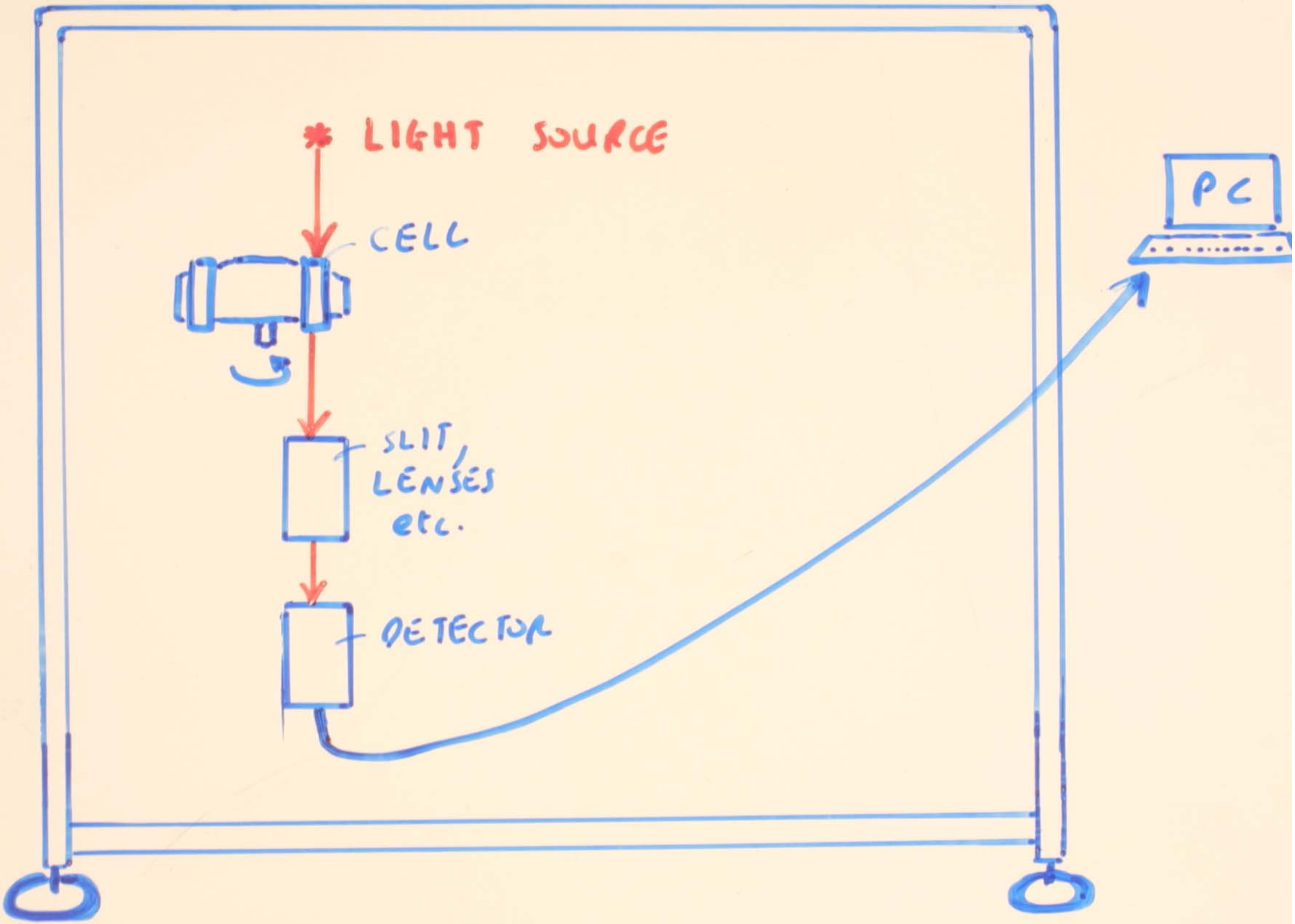
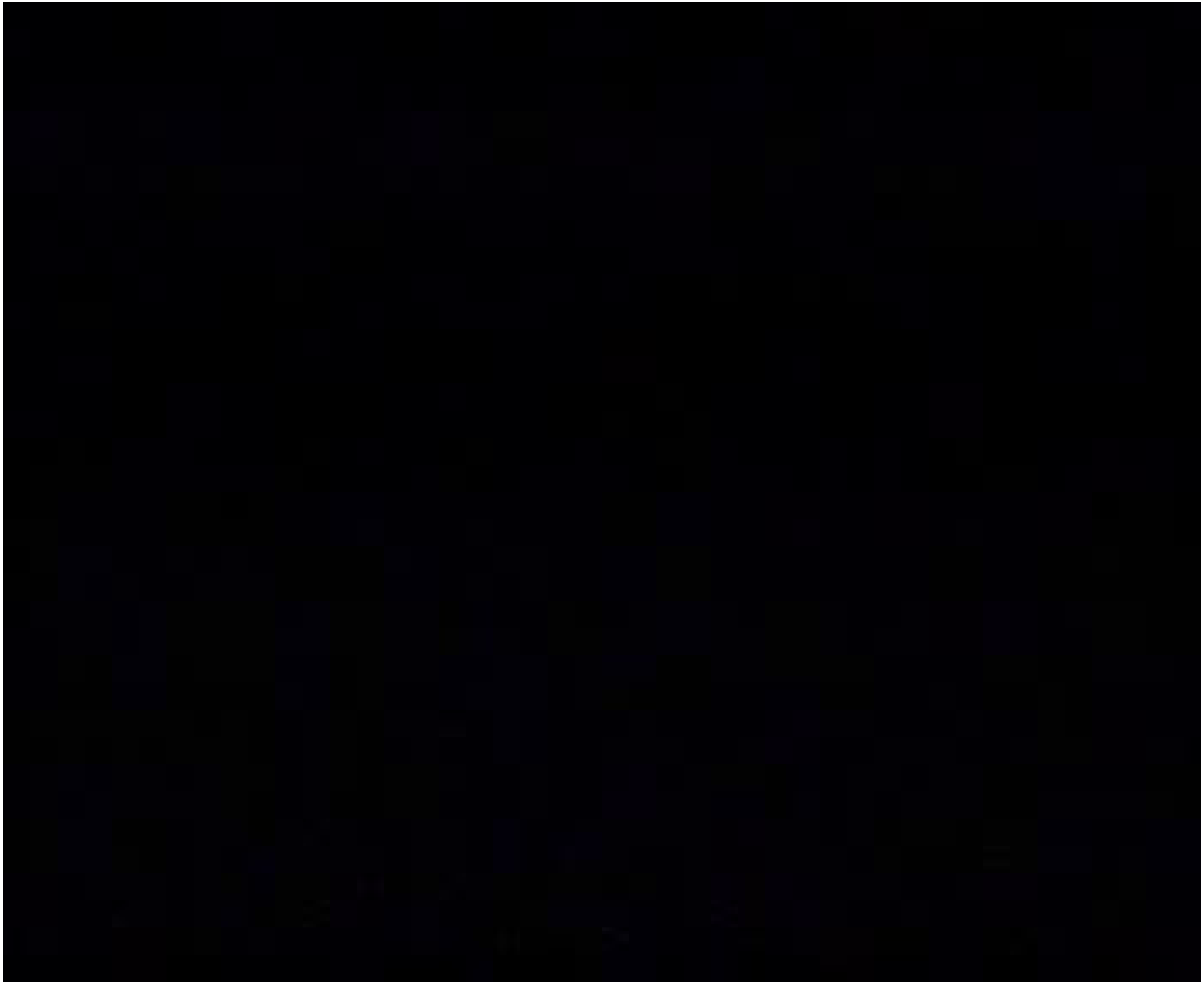
c (mg/ml)

Analytical Ultracentrifugation

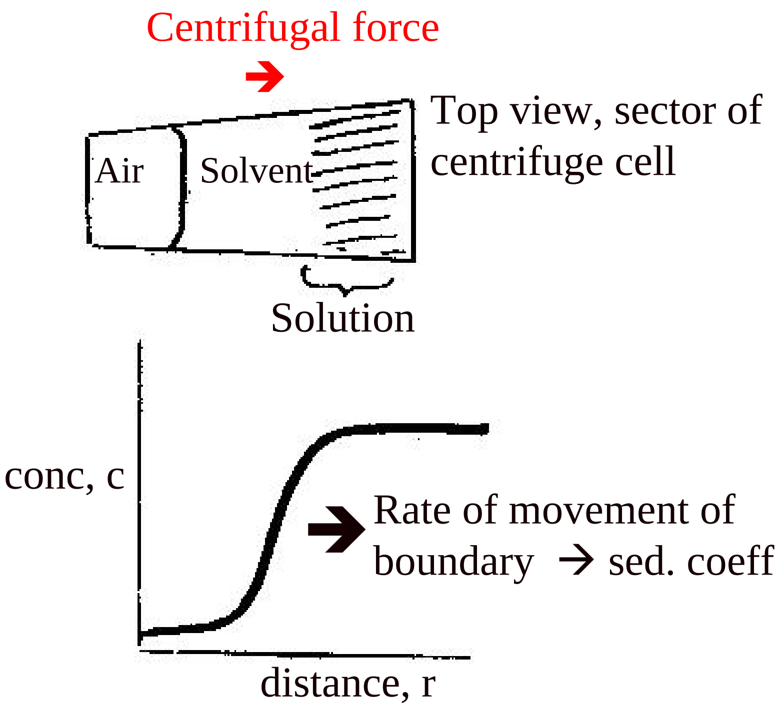


Optima XLA/ XLI


LIGHT SOURCE
CELL
SLIT, LENSES etc.
DETECTOR
PC



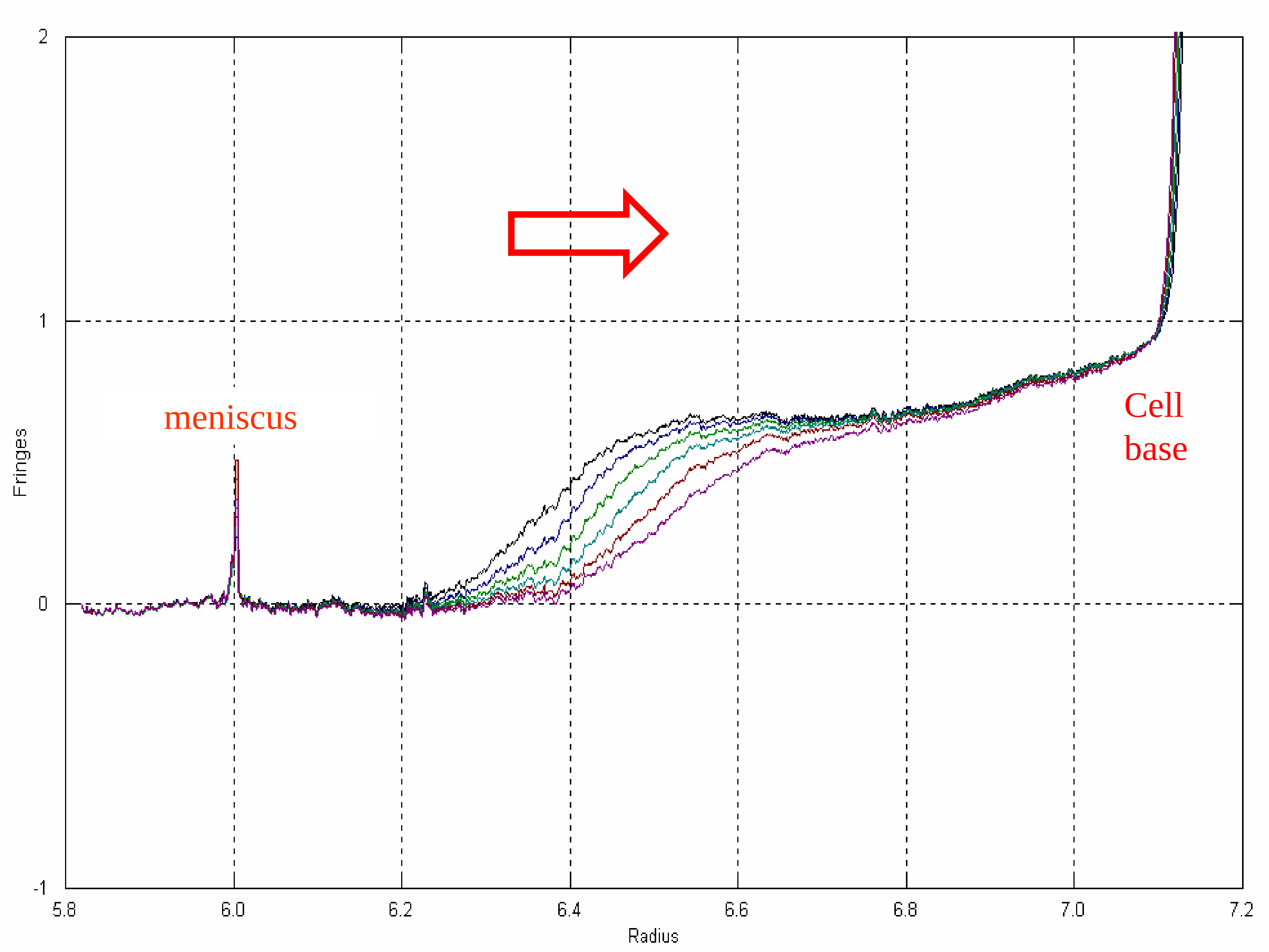
Sedimentation Velocity



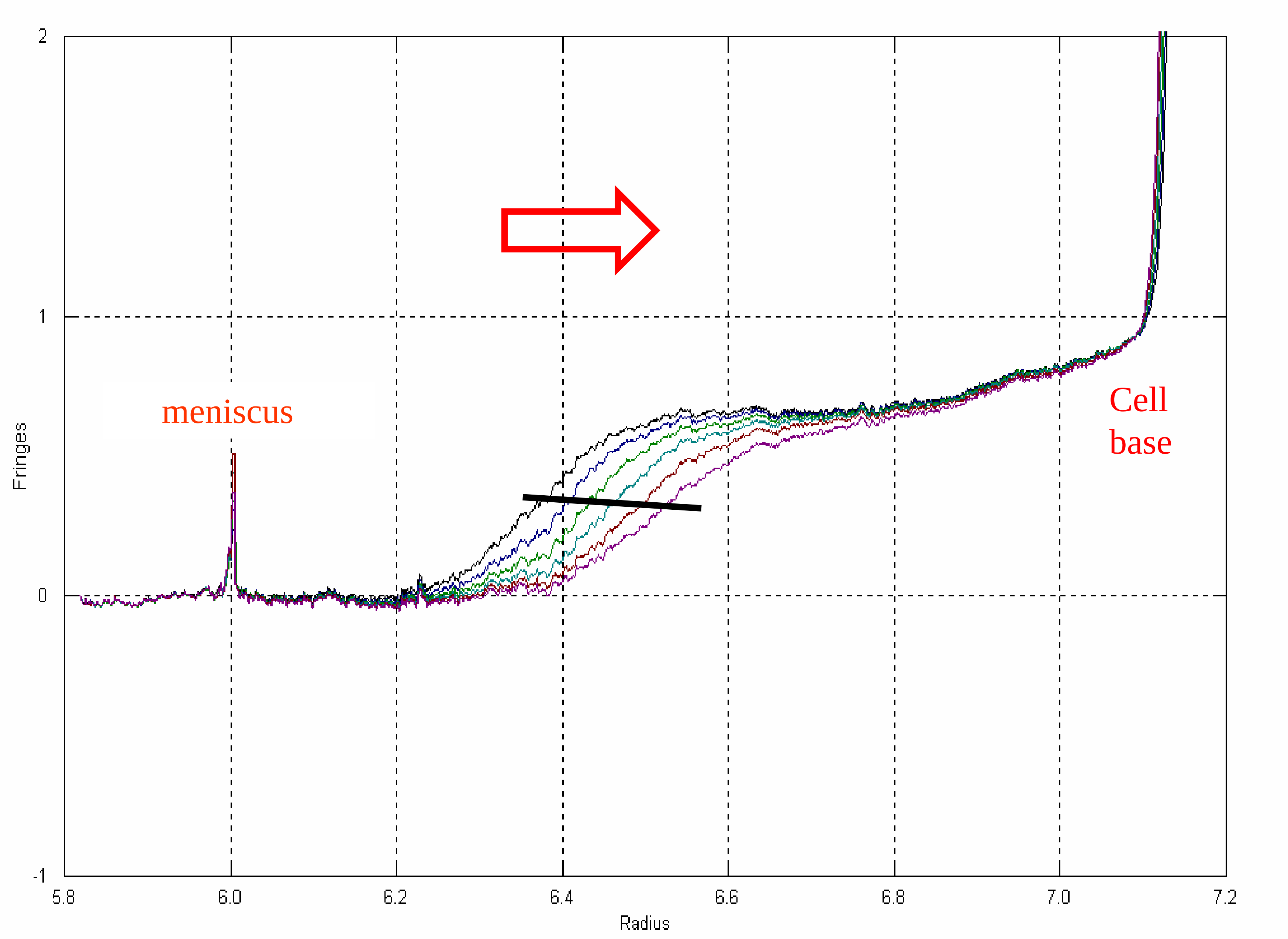
$\Rightarrow s^{o}_{20,w}$

Sedimentation coefficient, S

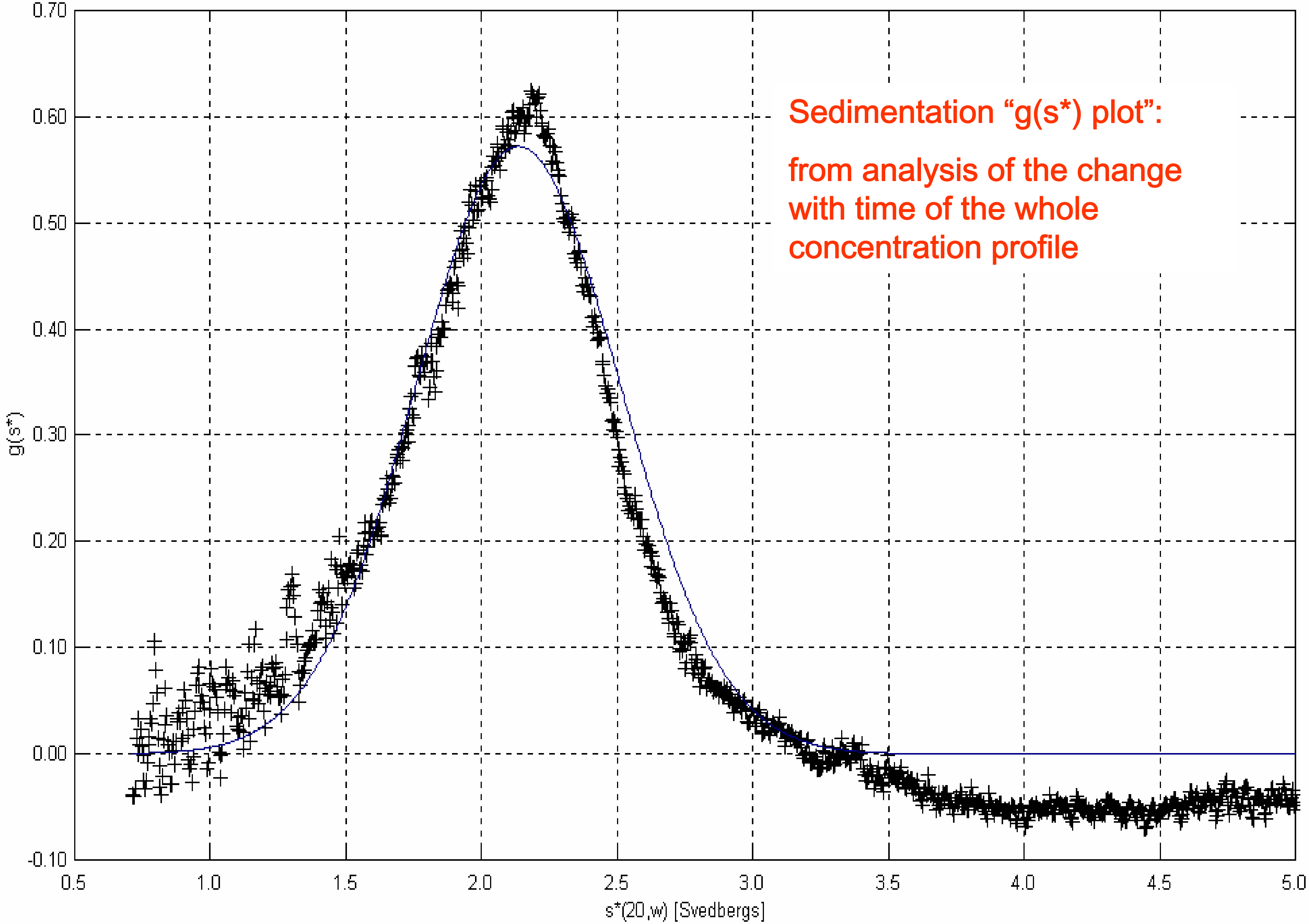
Chitosan G213, 0.5 mg/ml



Chitosan G213, 0.5 mg/ml



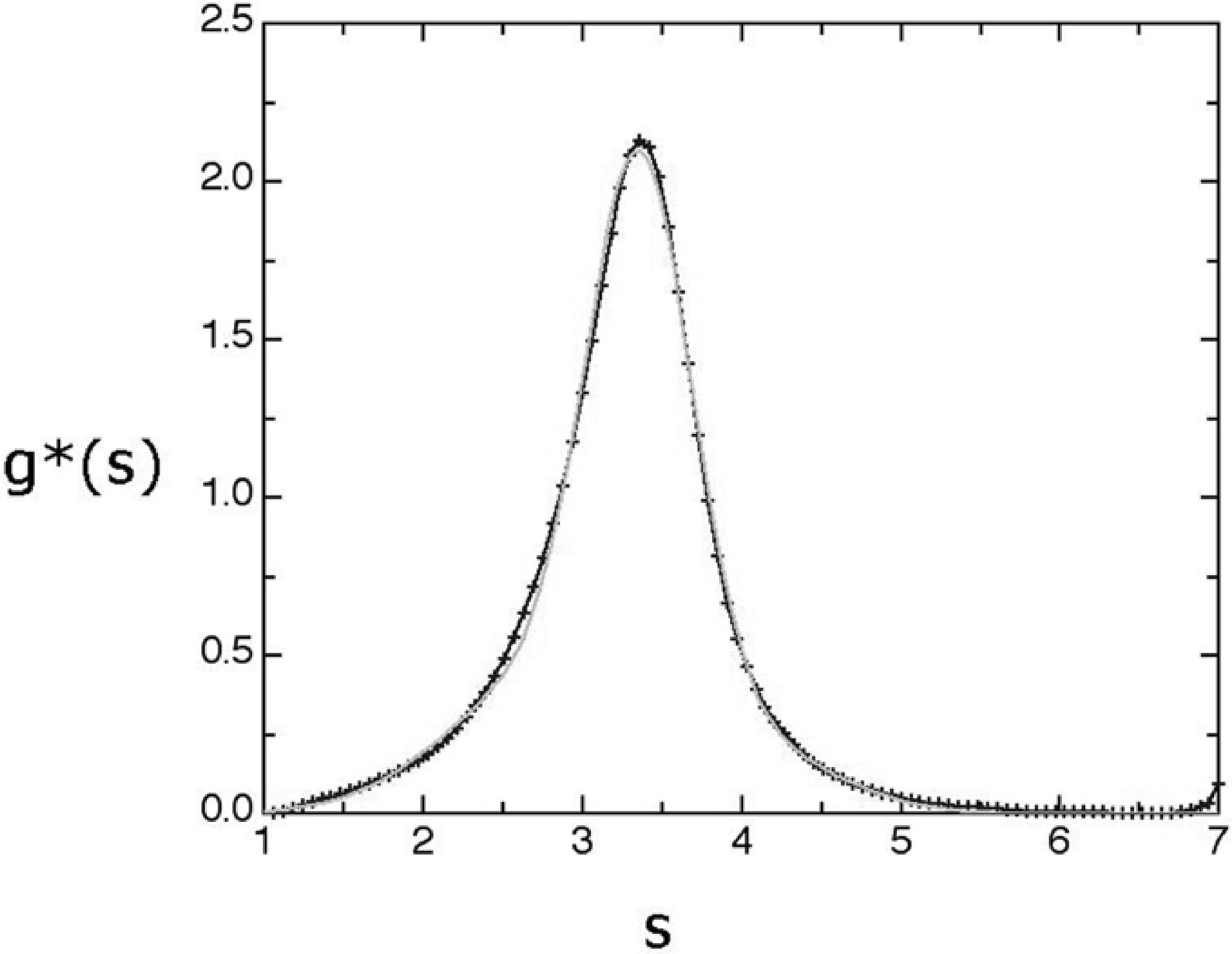
Chitosan G213, 0.5 mg/ml



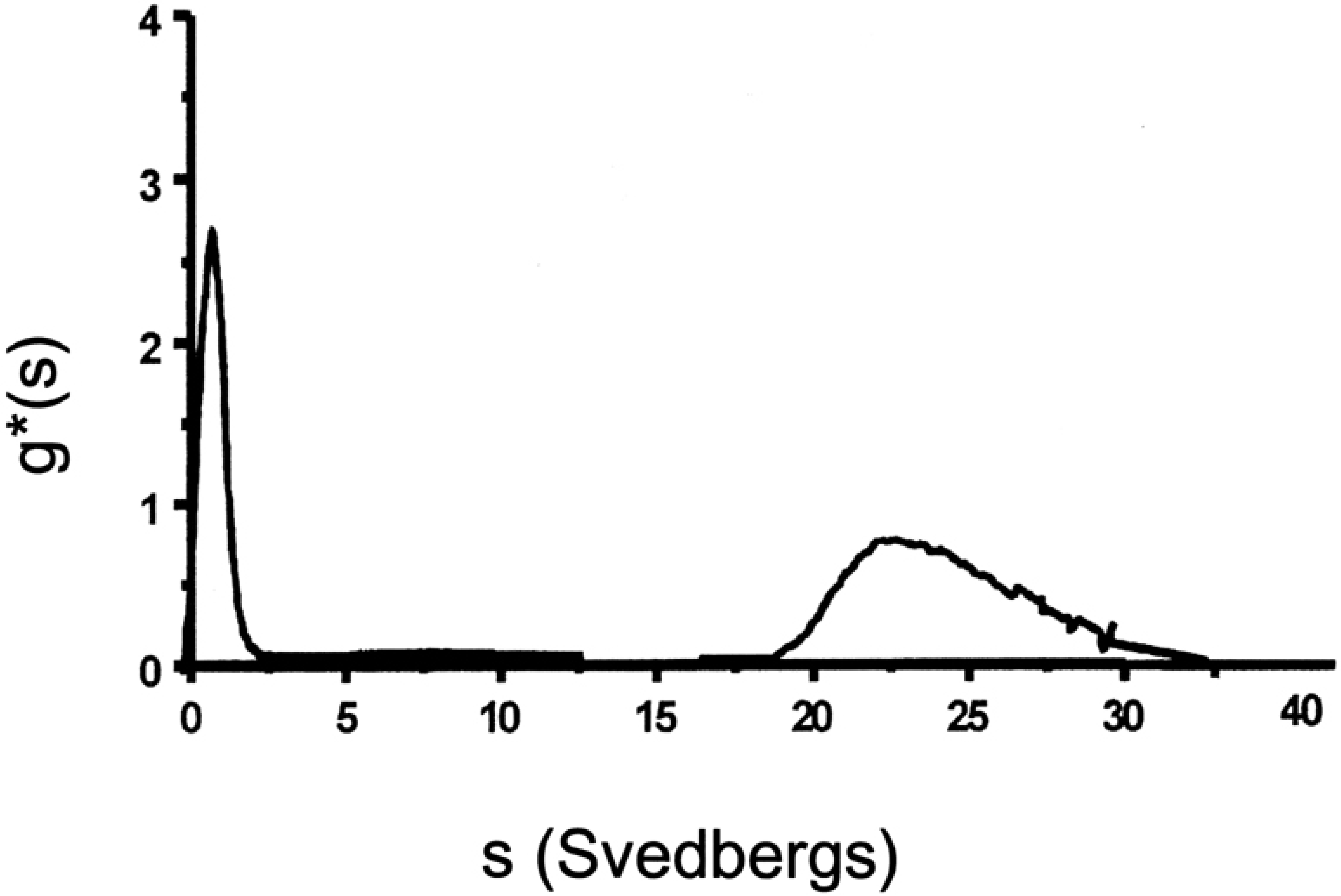
Sedimentation "g(s*) plot":

from analysis of the change with time of the whole concentration profile

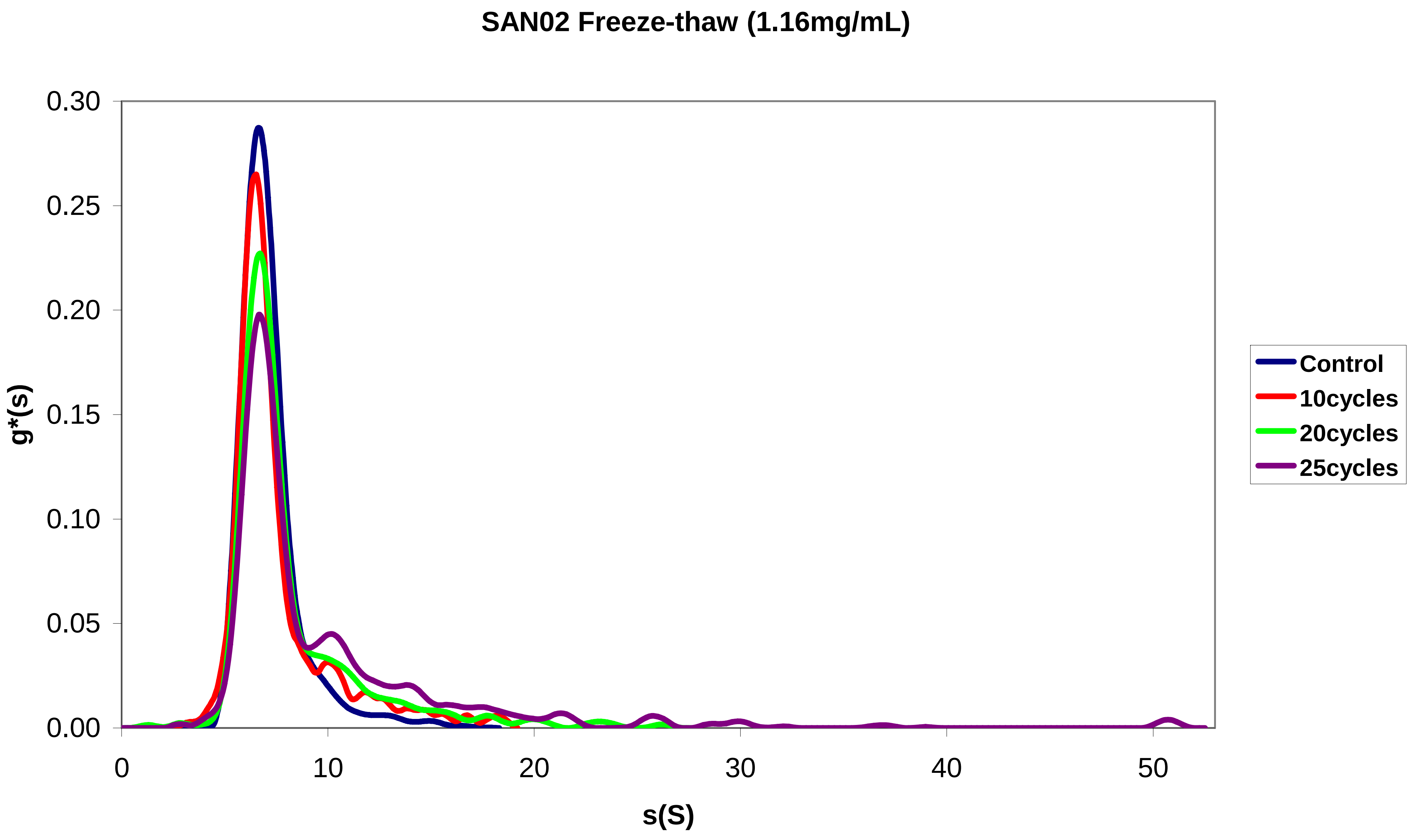
Guar, 0.75 mg/ml



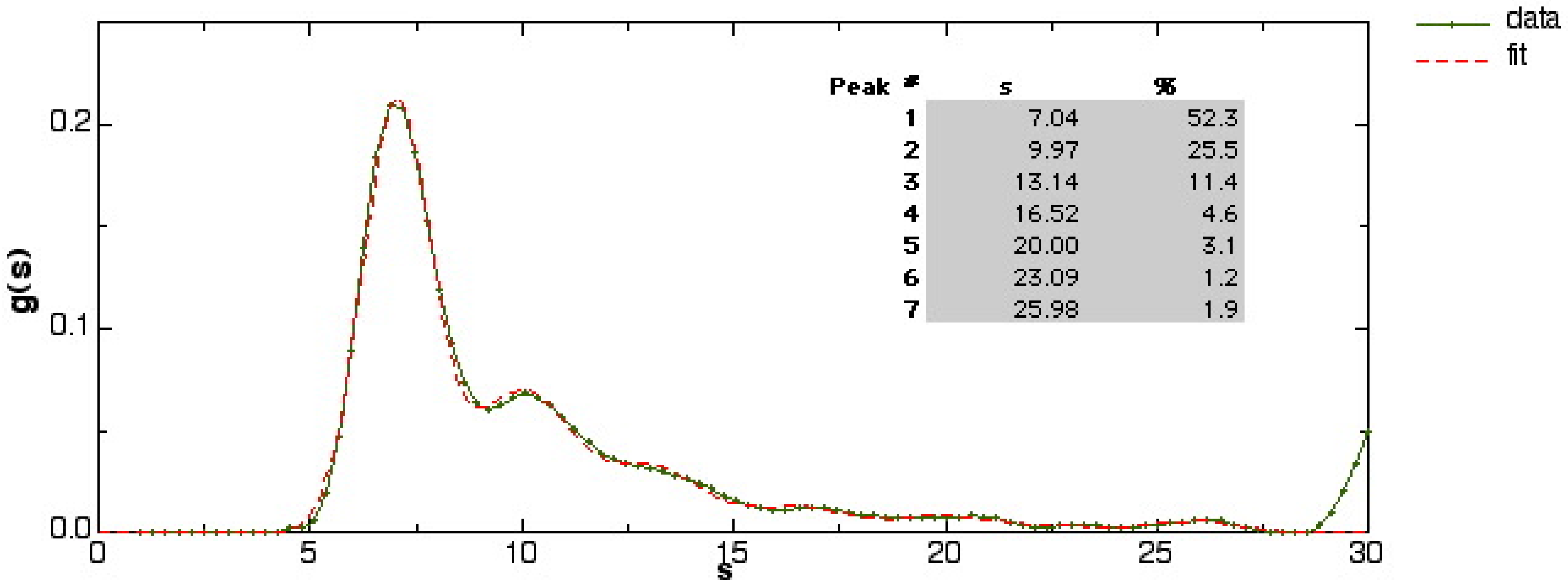
Wheat starch, 8 mg/ml



……stability studies



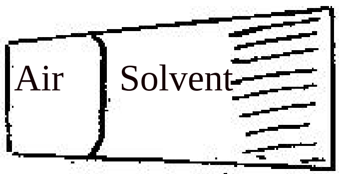
Multi-Gaussian fit estimates *proportions* of each species too:



2 types of AUC Experiment:

Sedimentation Velocity

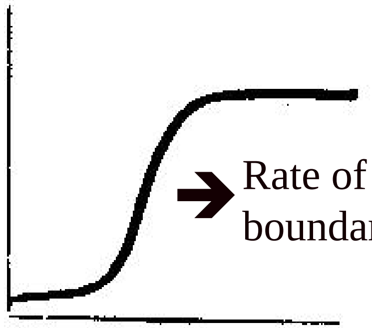
Centrifugal force →



Top view, sector of centrifuge cell

Solution

conc, c



→ Rate of movement of boundary → sed. coeff

distance, r

$s^{o}_{20,w}$

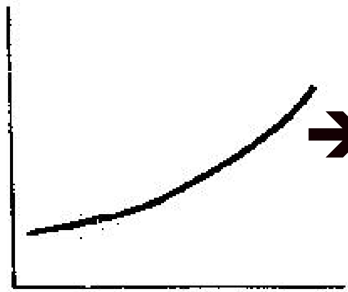
$1S = 10^{-13}$sec

Sedimentation Equilibrium

Centrifugal force →

← Diffusion

conc, c

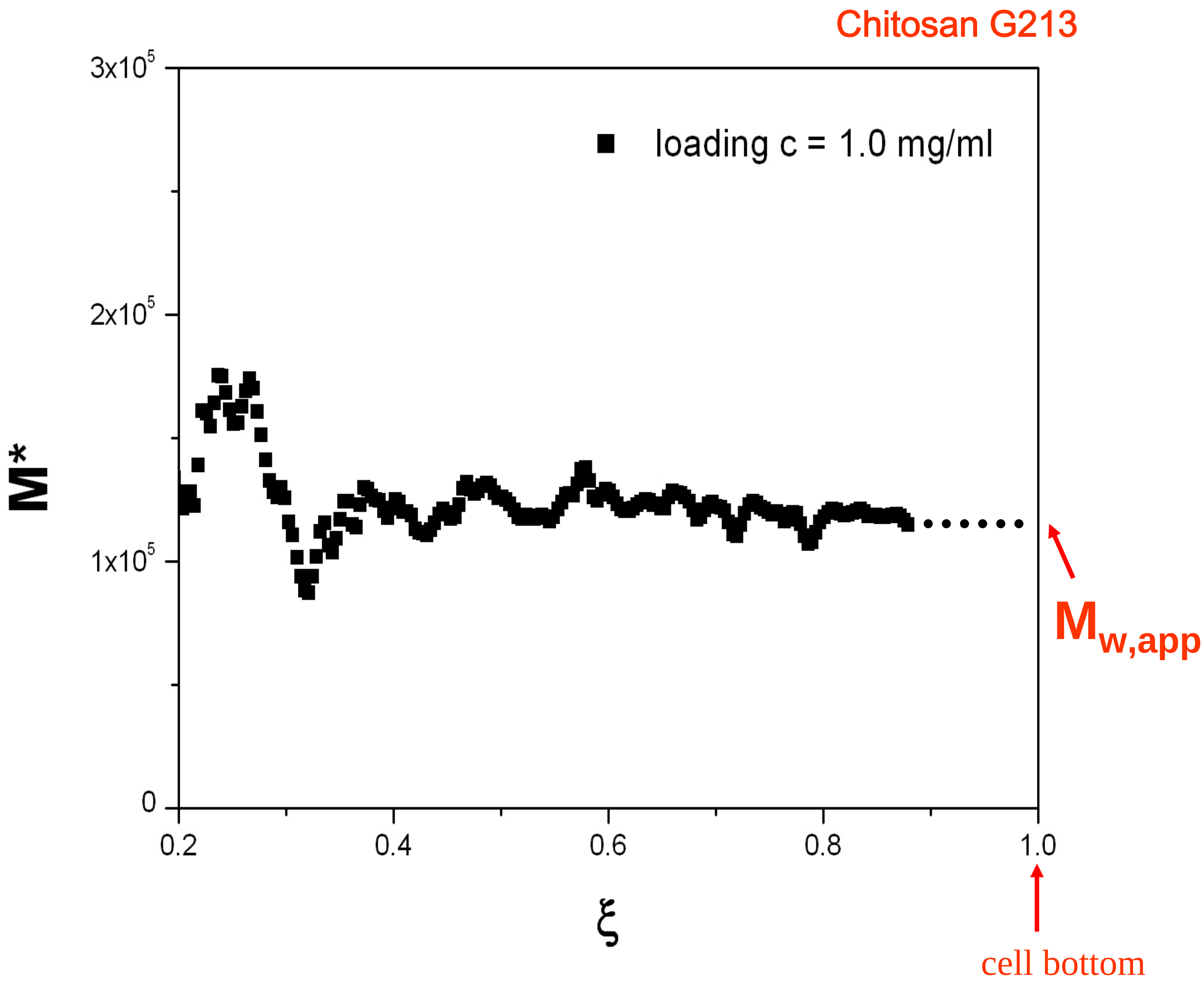


→ STEADY STATE PATTERN

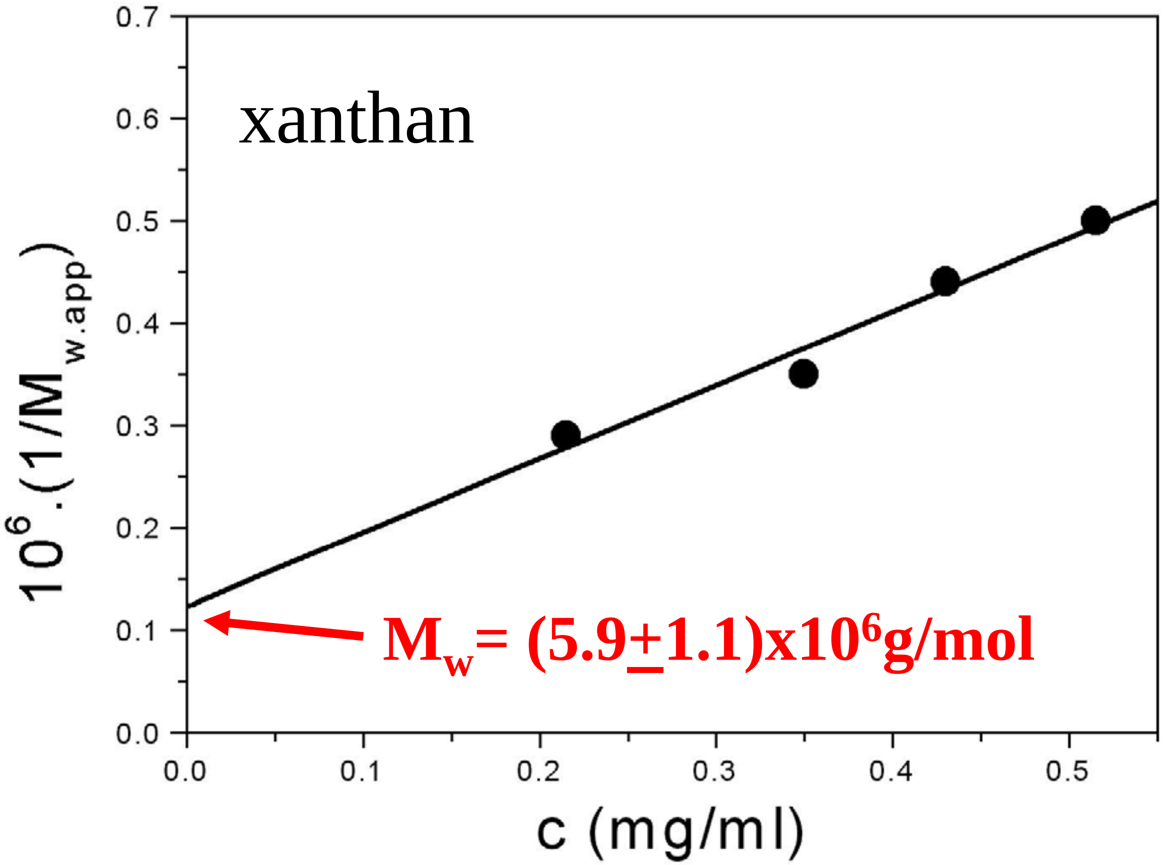
distance, r

FUNCTION ONLY OF MOL. WEIGHT PARAMETERS

Extraction of $M_{w,app}$ from sedimentation equilibrium and "MSTAR" analysis



Extraction of $M_{w,app}$ from sedimentation equilibrium and "MSTAR" analysis



3. Conformation in solution

Conformation in solution

1. Experimental data required

- Molecular weight
- $[\eta]$, s, R_g

2. Modelling strategies

- General conformation type (rod, coil or sphere etc.)
- Measure of flexibility – the persistence length, L_p,
- If ~ rigid then aspect ratio.

Conformation in solution

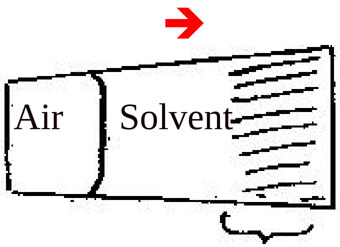
1. Experimental data required

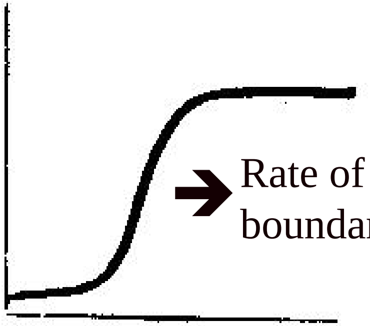
- Molecular weight: SEC-MALLs reinforced by sed. equilibrium
- [η], *s*, R_g

2. Modelling strategies

- General conformation type (rod, coil or sphere etc.)
- Measure of flexibility – the persistence length, L_p,
- If ~ rigid then aspect ratio.

Sedimentation Velocity

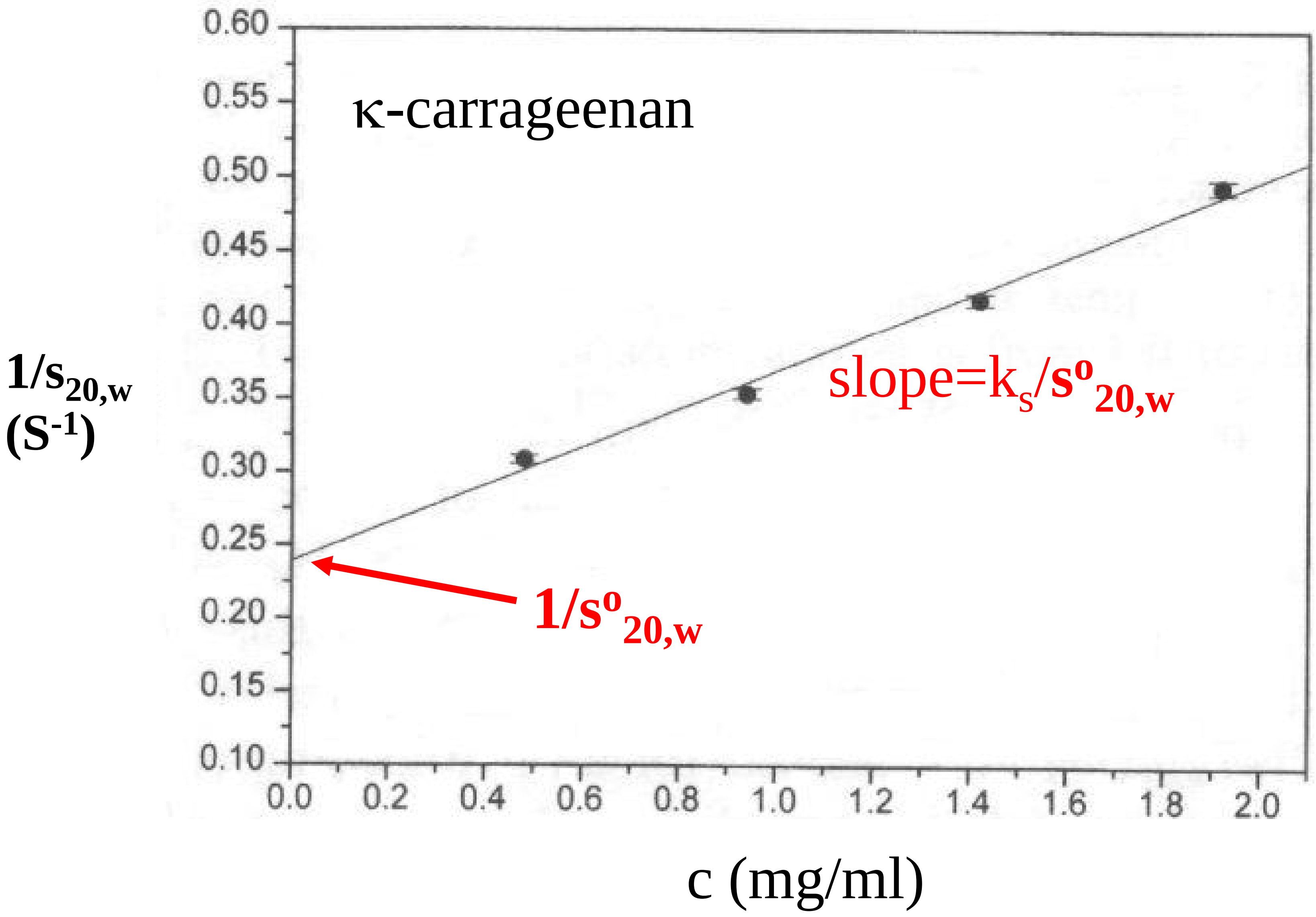




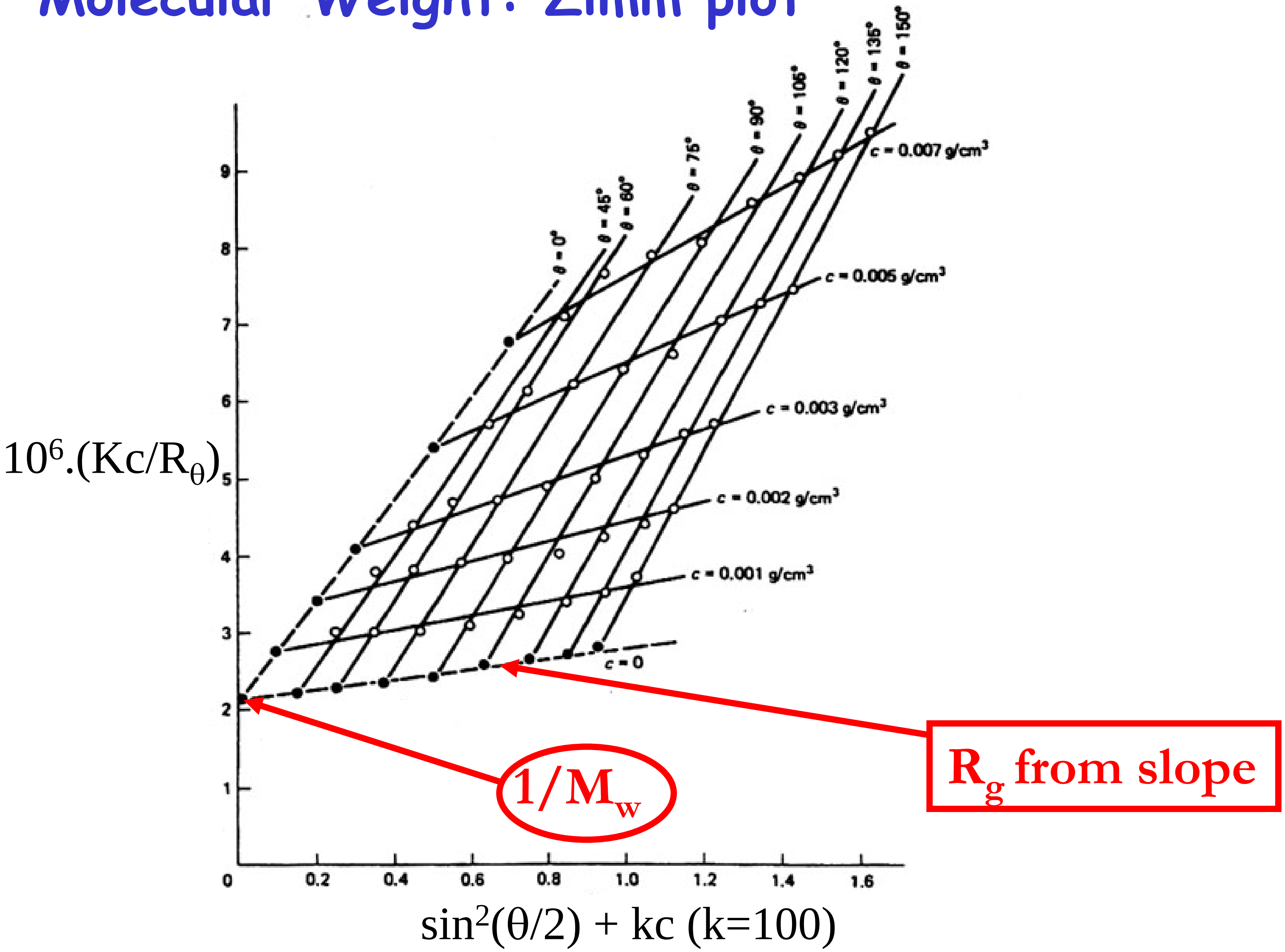
⇨ $s^{o}_{20,w}$

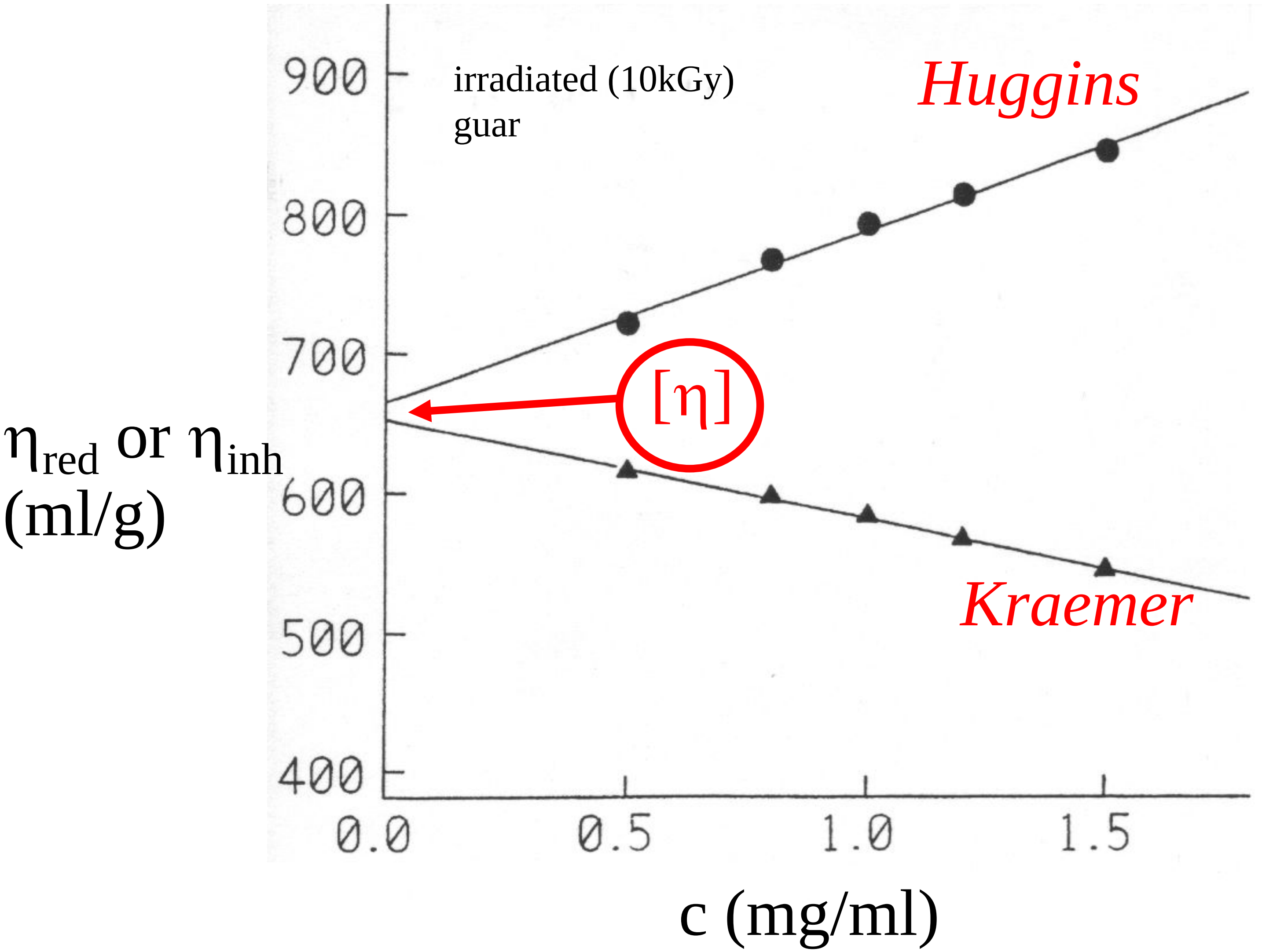
Sedimentation coefficient, S

$s^o_{20,w}$ extraction



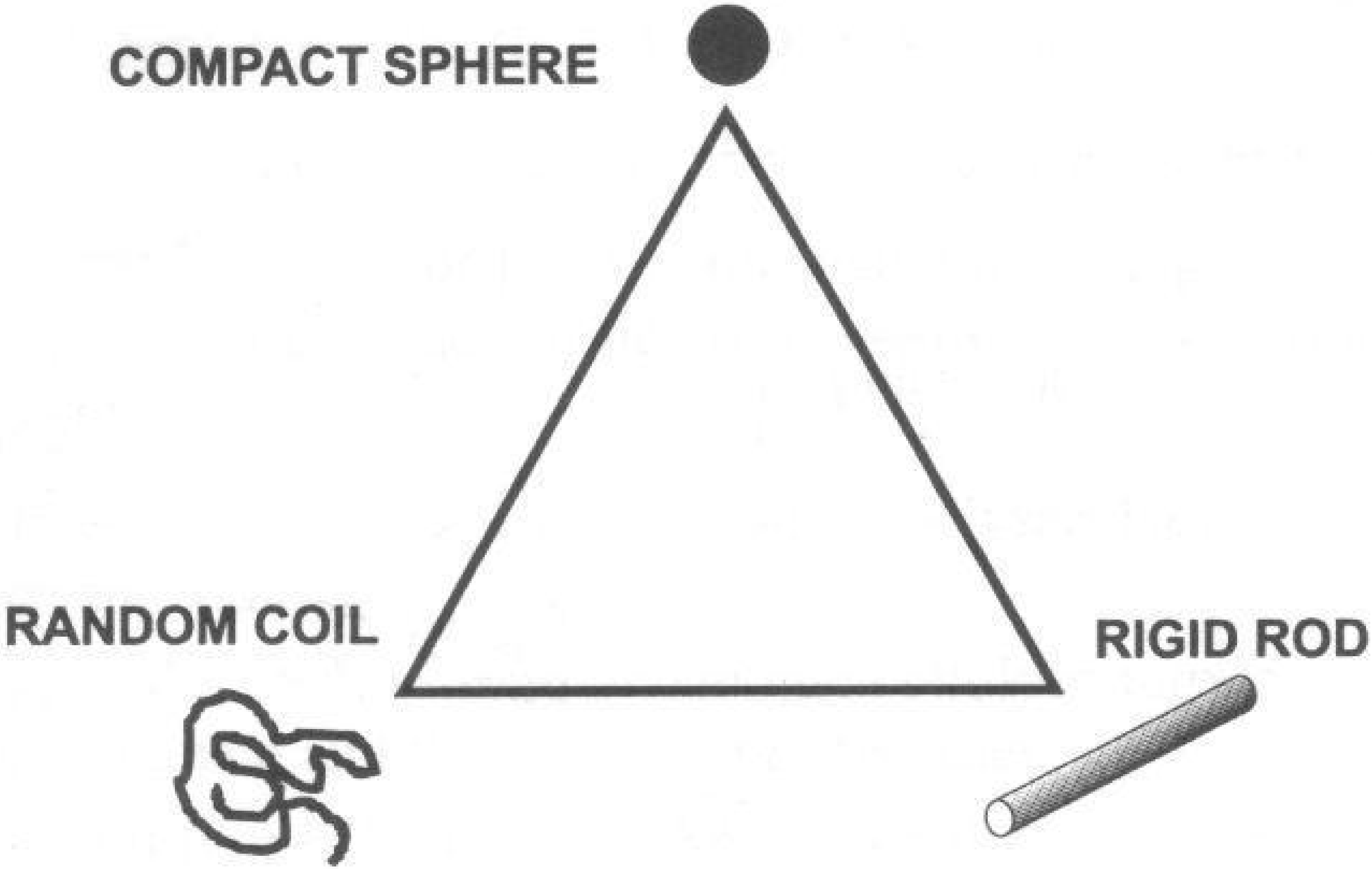
Molecular Weight: Zimm plot

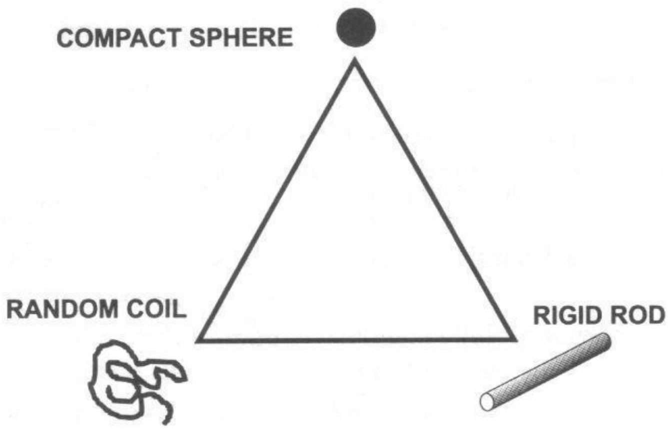




irradiated (10kGy)
guar
Huggins
Kraemer
[η]
900
800
700
600
500
400
0.0
0.5
1.0
1.5
η_{red} or η_{inh}
(ml/g)
c (mg/ml)

Haug Triangle

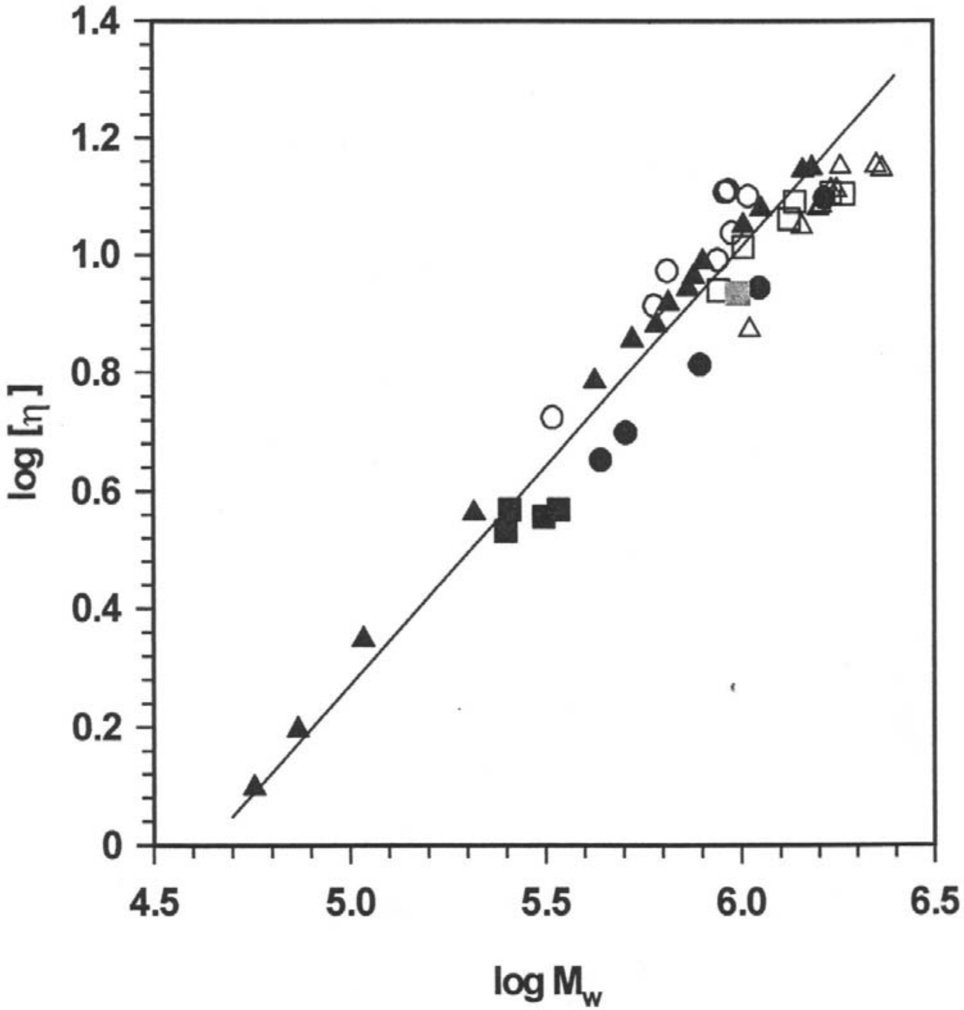




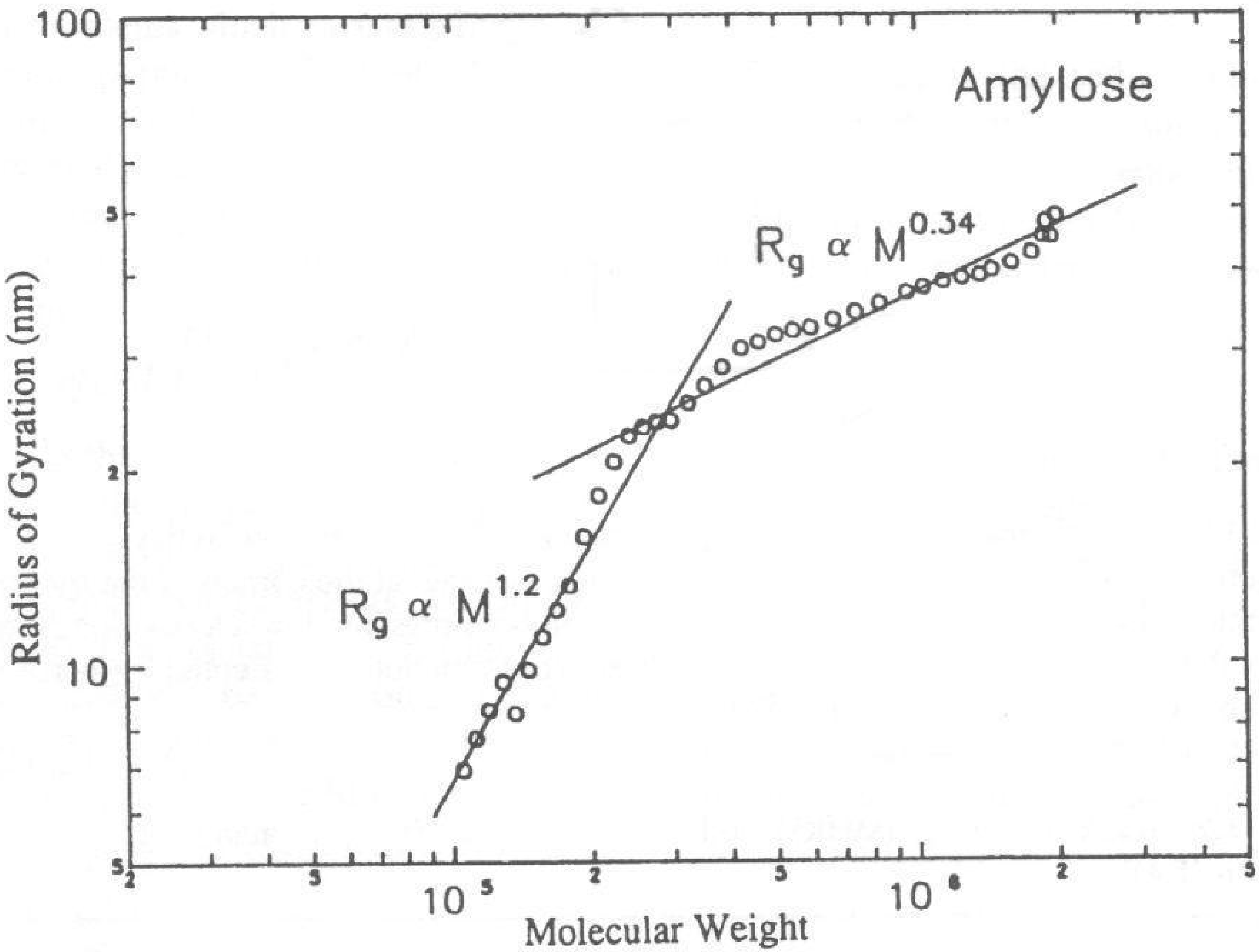
Sphere	Rod	Coil
$[\eta] \sim M^{0}$	$[\eta] \sim M^{1.8}$	$[\eta] \sim M^{0.5\text{-}0.8}$
$s^{o}_{20,w} \sim M^{0.67}$	$s^{o}_{20,w} \sim M^{0.15}$	$s^{o}_{20,w} \sim M^{0.4\text{-}0.5}$
$R_g \sim M^{0.33}$	$R_g \sim M^{1.0}$	$R_g \sim M^{0.5\text{-}0.6}$

Mark-Houwink-Kuhn-Sakurada
Power law plot

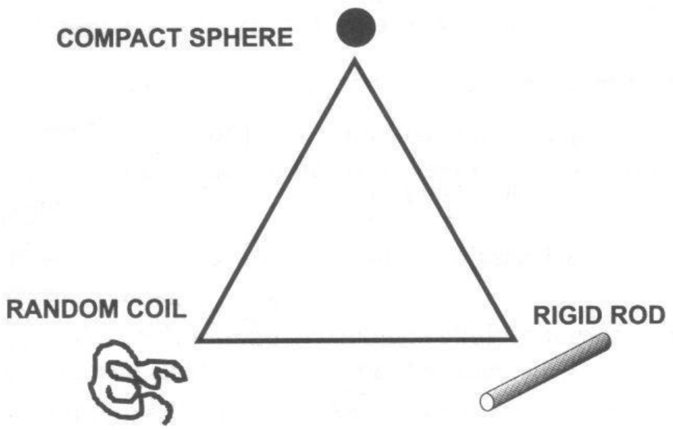
Galactomannans
a=0.74±0.01



Change in Conformation



Rollings, 1992



Sphere	Rod	Coil
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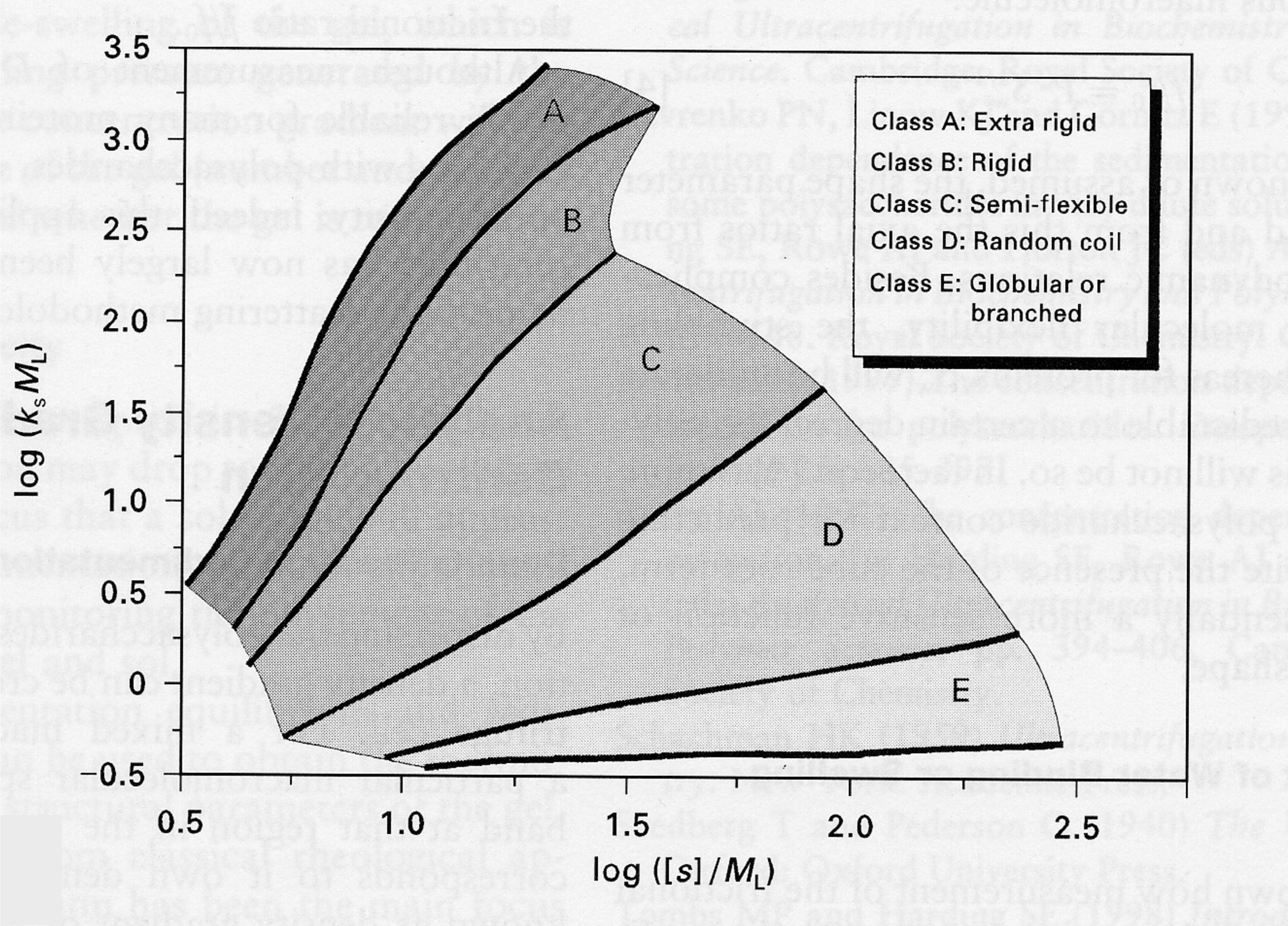
$s^{o}_{20,w} \sim M^{0.67}$	$s^{o}_{20,w} \sim M^{0.15}$	$s^{o}_{20,w} \sim M^{0.4\text{-}0.5}$
$R_g \sim M^{0.33}$	$R_g \sim M^{1.0}$	$R_g \sim M^{0.5\text{-}0.6}$
$k_s/[\eta] \sim 1.6$	$k_s/[\eta] < 1$	$k_s/[\eta] \sim 1.6$

Conformation Zoning:



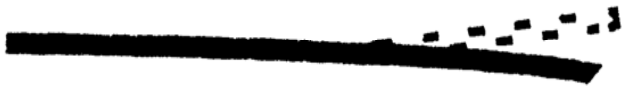
Pavlov, Harding & Rowe., 1997

Conformation Zoning:

ZONE A

Extra-rigid Rod: Schizophyllan

ZONE B



Rigid-rod: DNA

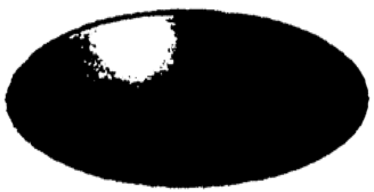
ZONE C

Semi-flexible coil: cellulose derivatives, chitosans

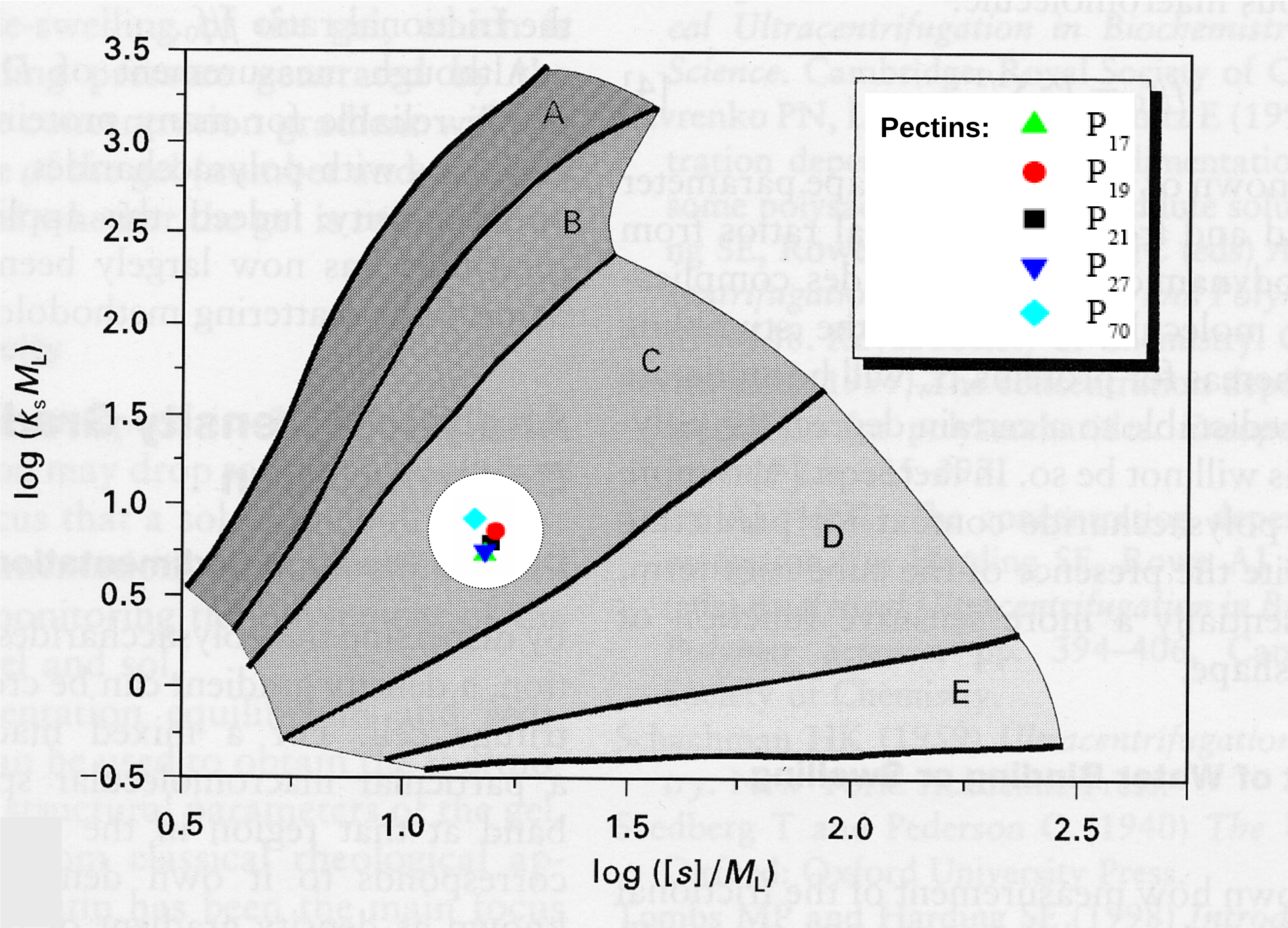
ZONE D

Random coil: Dextran, glycoproteins from mucus

ZONE E



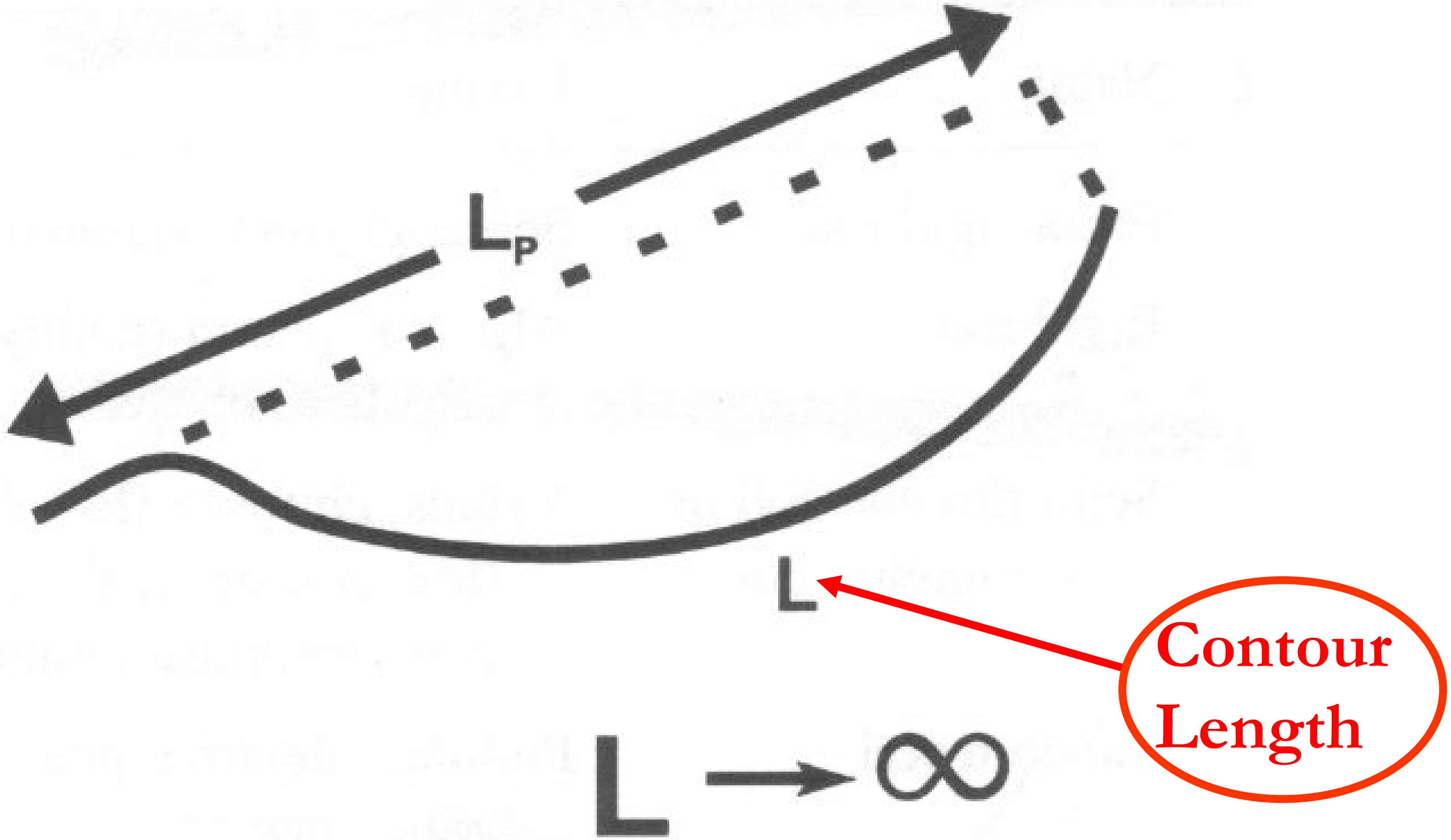
Globular/Branched: Proteins/glycogen



Morris et al., 2007

Worm-like Chain

Flexibility parameter: Persistence length L_p



Kuhn-statistical length $\lambda^{-1} = 2L_p$

Worm-like Chain

Flexibility parameter: Persistence length L_p

Theoretical limits: Random coil $L_p = 0$

Rigid rod L_p = infinity

Practical limits: Random coil $L_p \sim$ 1-2nm

Rigid rod $L_p \sim$ 200nm

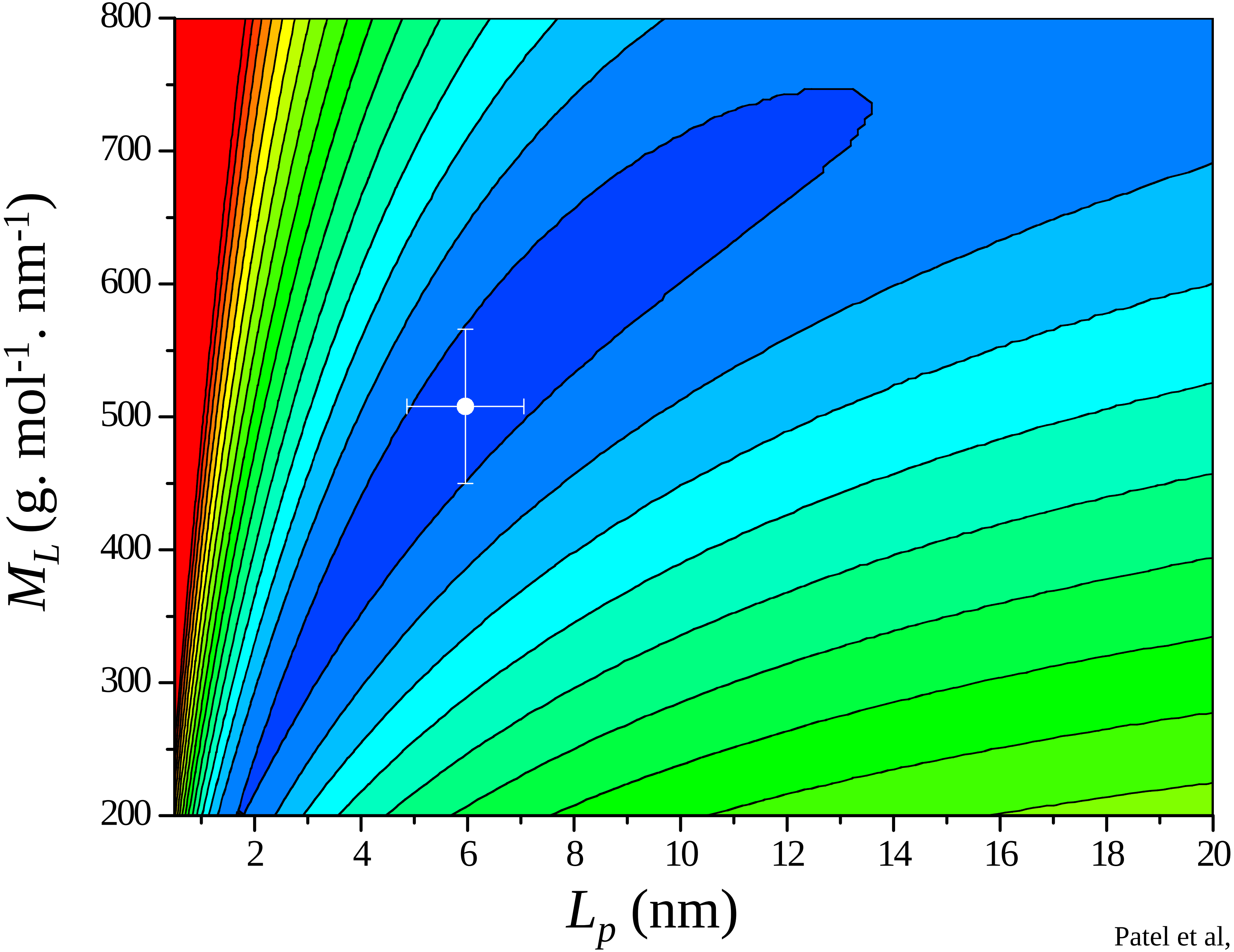
“Bohdanecky” relation

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3} \left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2}$$

“Yamakawa-Fujii” relation

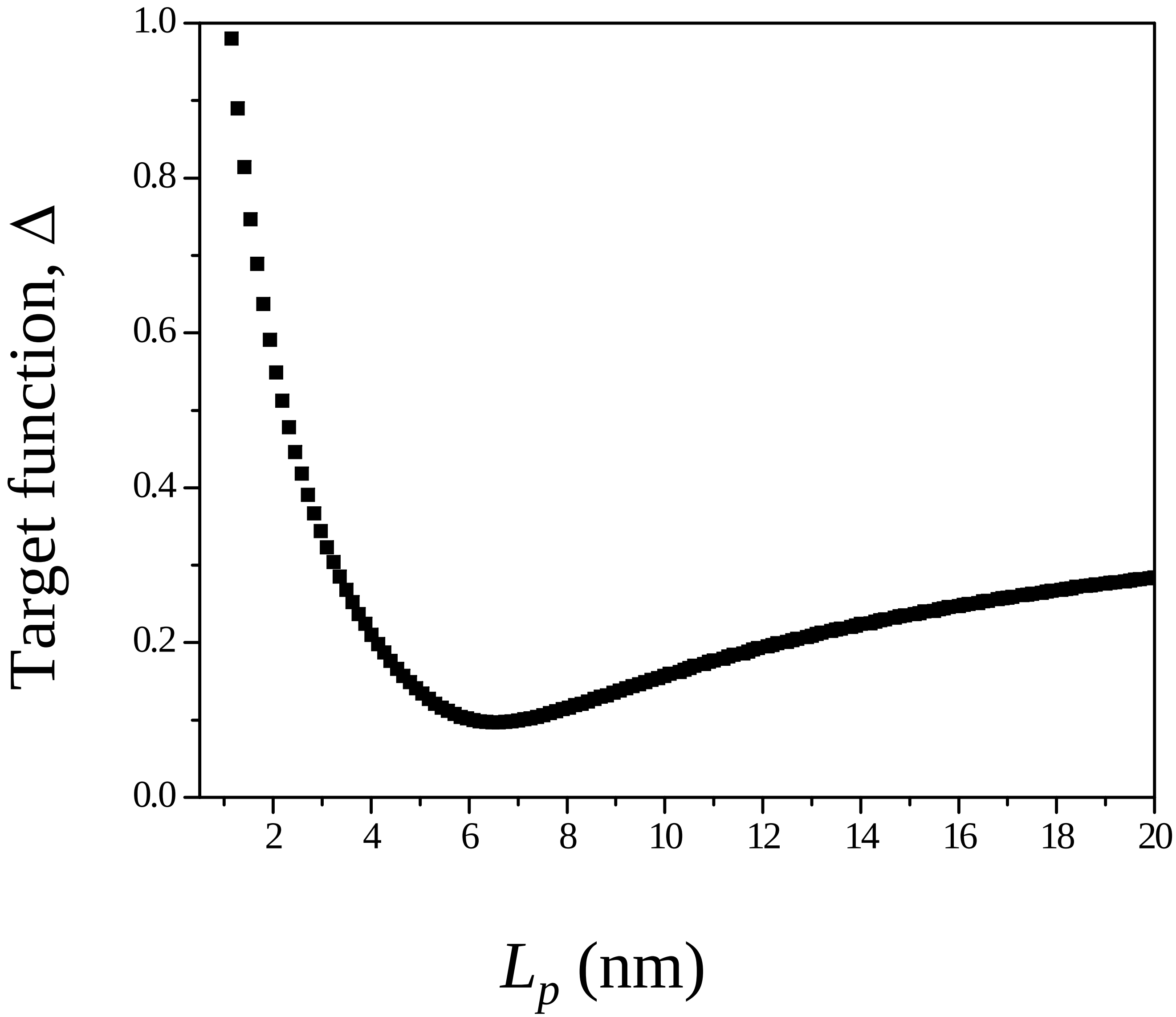
$$s^0 = \frac{M_L\left(1-\bar{v}\rho_0\right)}{3\pi\eta_0 N_A} \times \left[1.843\left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3\left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + ...\right]$$

Global plot: xyloglucan



Patel et al, 2007

….or if you know the mass per unit length



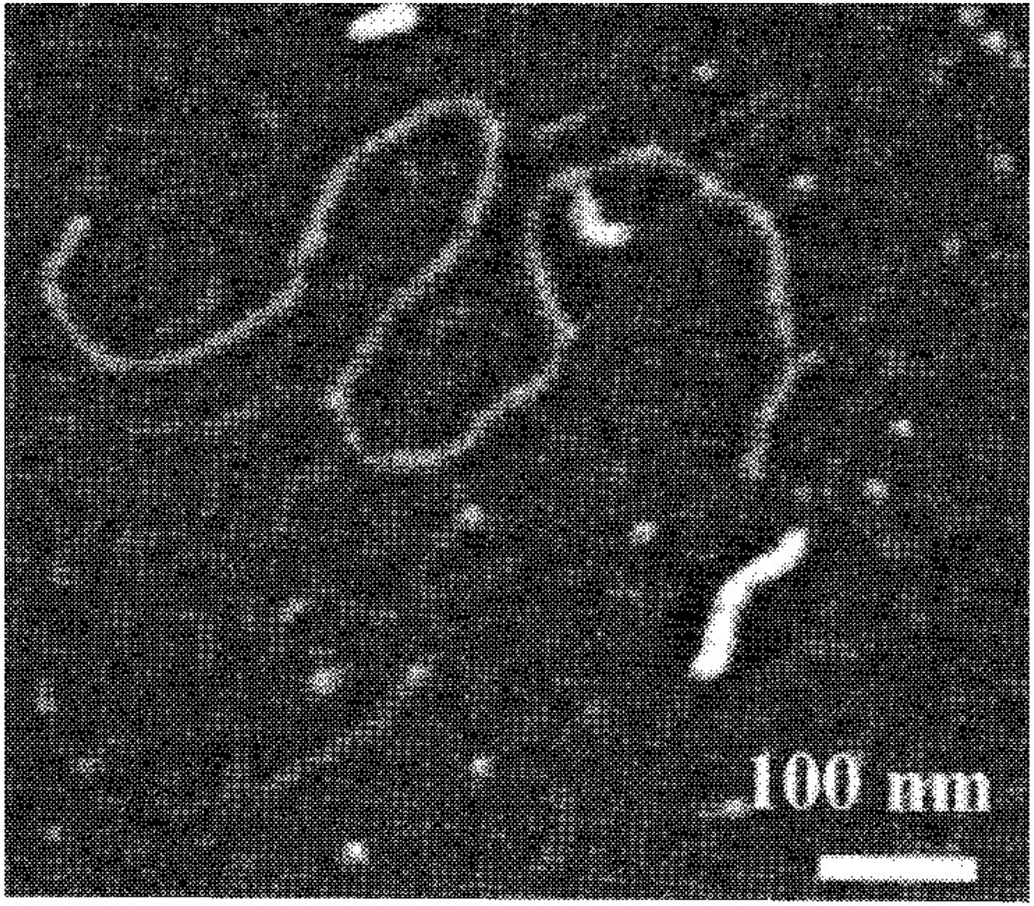
Patel et al, 2007

Flexibilities of carbohydrate polymers

Carbohydrate Polymer	L_p (nm)
Pullulan	1-2
Xyloglucan	5-8
Pectins	10-20
DNA	45
Schizophyllan	120-200
Scleroglucan	180 ± 30
Xanthan	200

4. Interactions

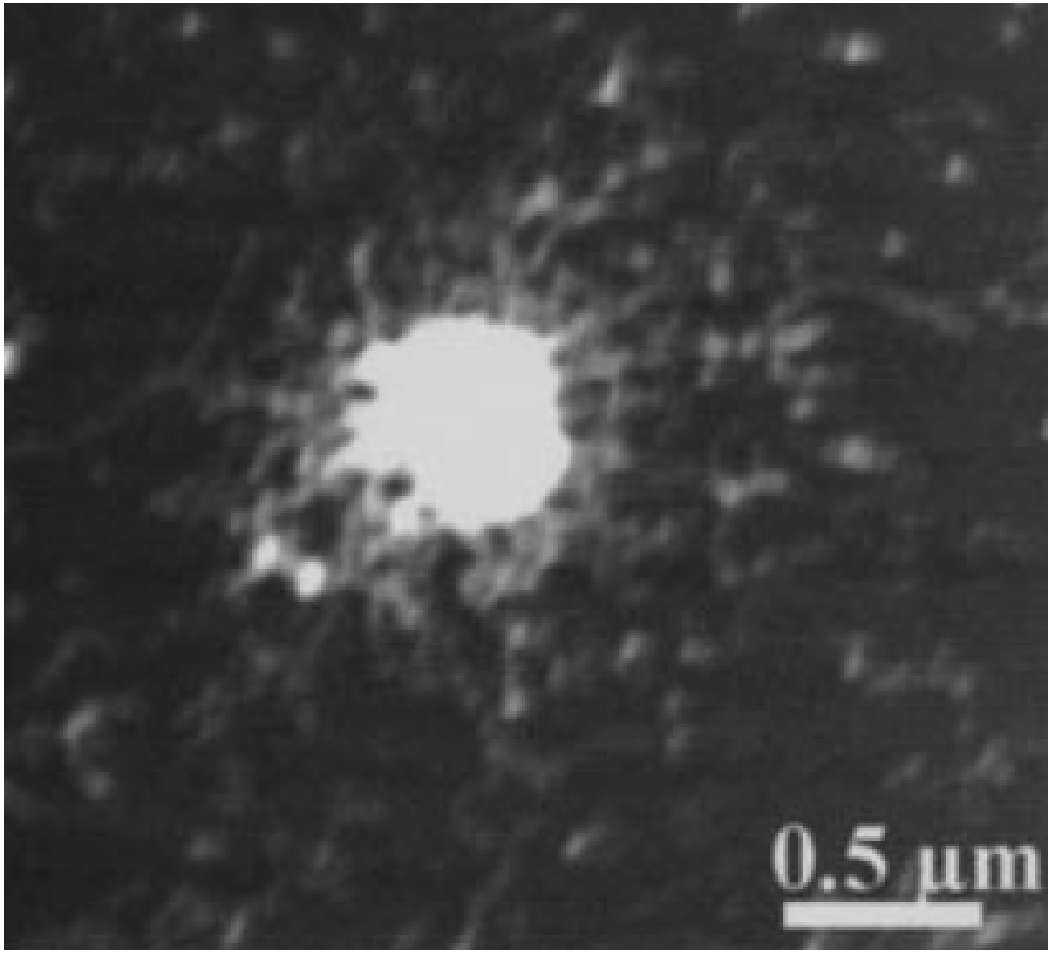
Atomic Force Microscopy: chitosan



Sedimentation coefficient $s^o_{20,w} \sim 1S$

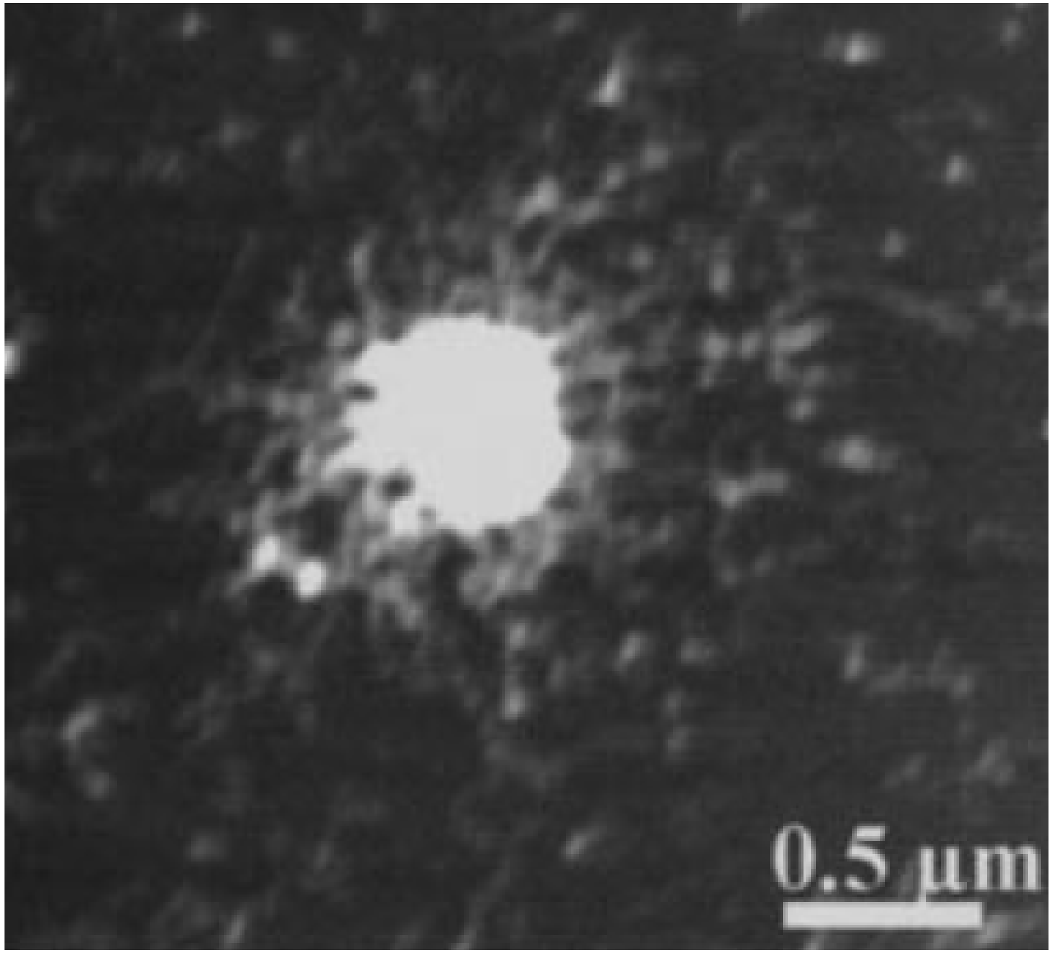
Deacon et al, 2000

chitosan-mucin complex



Sedimentation coefficient $s^o_{20,w}$ ~ 2000S

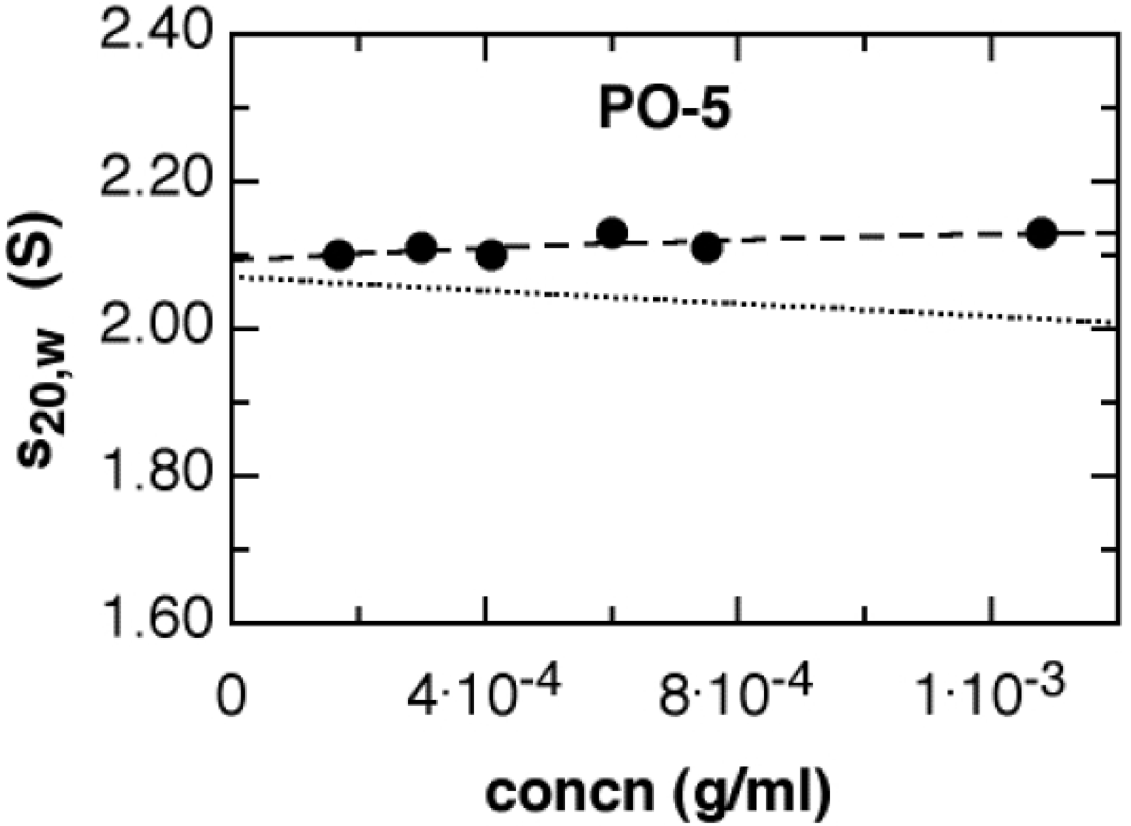
chitosan-mucin complex



Sedimentation coefficient $s^{o}_{20,w}$ ~ 2000S

very strong, irreversible interaction

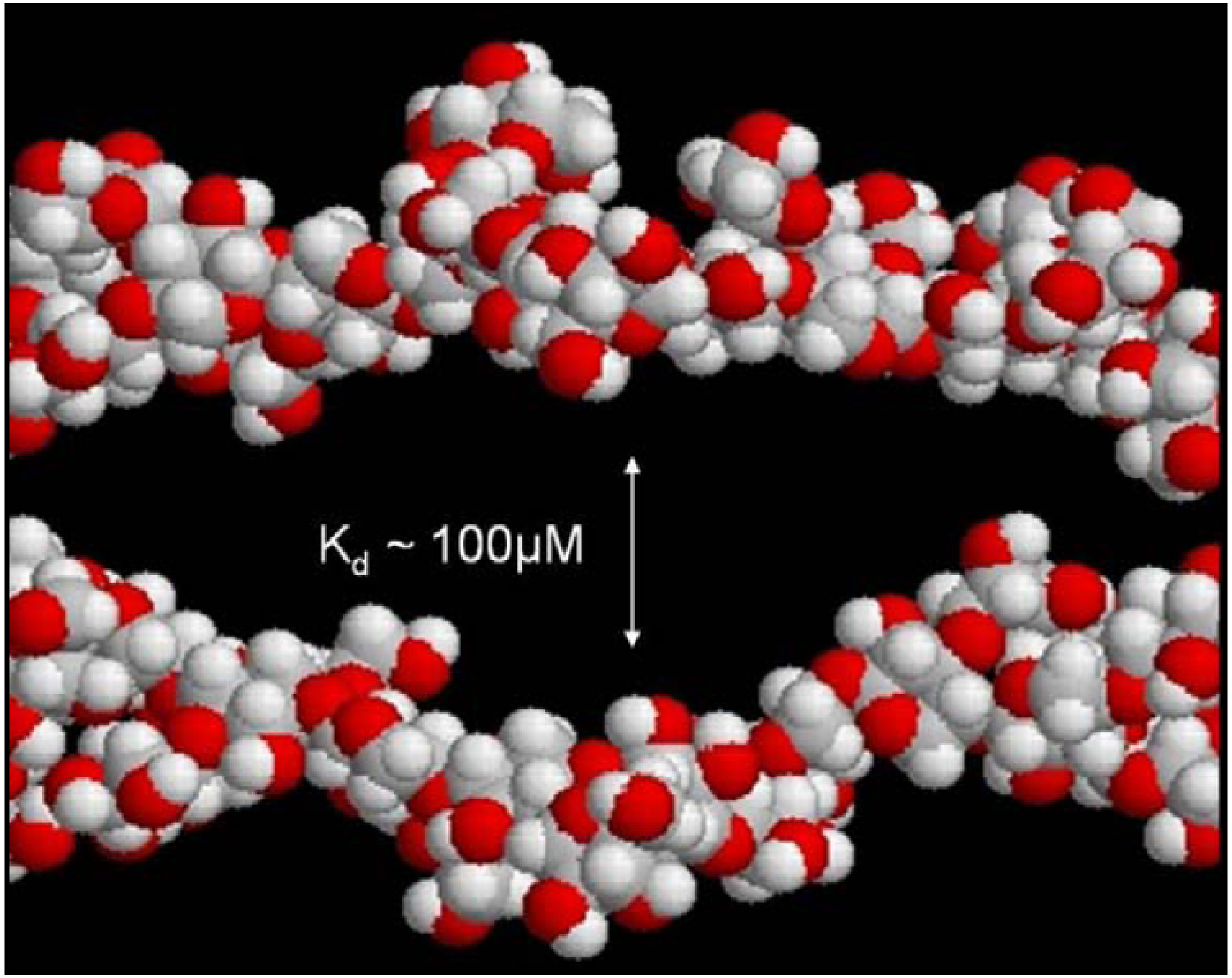
“bioactive” arabinoxylan



very weak, reversible interaction

Patel et al, 2007

"bioactive" arabinoxylan



very weak, reversible interaction

Physical characterisation

1. Viscosity, stability
2. Heterogeneity, Molecular weight & distribution
3. Conformation in solution
4. Interactions

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for a copy of this presentation …

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