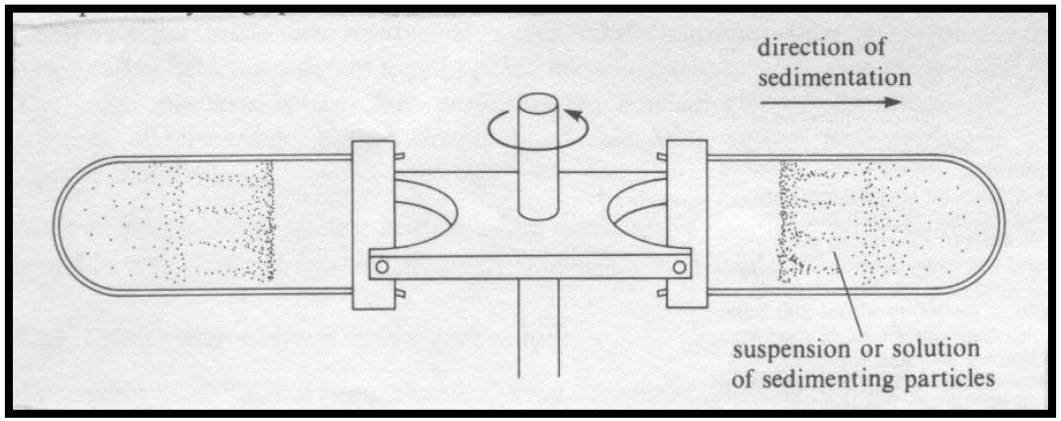
Datum/Zeit	Veranstaltungsort	Thema
Mo, 08.02.2010 10.00-11.30	Hörsaal Institut für Glaschemie Fraunhoferstrasse 6	***Albert Einstein and the Viscosity of Macromolecules***
Mo, 08.02.2010 12.15-13.45	Hörsaal Haus 1,IAAC, August-Bebel-Str. 2	***Light Scattering and SEC-MALLs***
Di, 09.02.2010 12.15-13.45	Institut für Materialwissenschaft und Werkstofftechnologie, HS 124 Löbdergraben 32	***Dynamic Light Scattering***
Mi, 10.02.2010 16.15-17.45	Hörsaal 3 Carl-Zeiss-Str. 3	***Analytical Ultracentrifugation I***
Do, 11.02.2010 14.15-15.45	Döbereiner Hörsaal	***Analytical Ultracentrifugation II: Interactions***

Lecture 4. Analytical Ultracentrifugation I: Molecular weight and conformation



Steve Harding

NCMH

The University of Nottingham

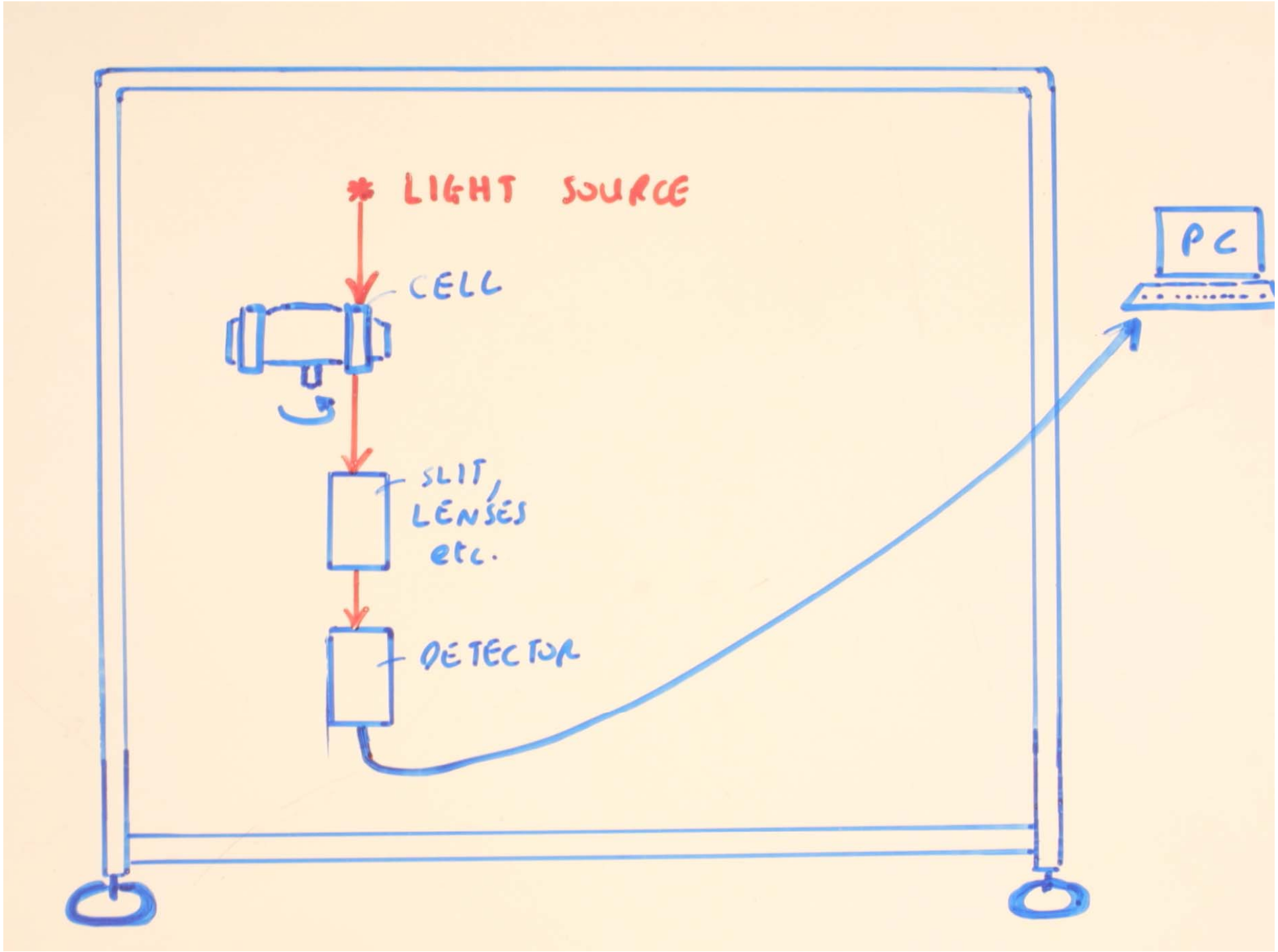
Analytical Ultracentrifugation Conference

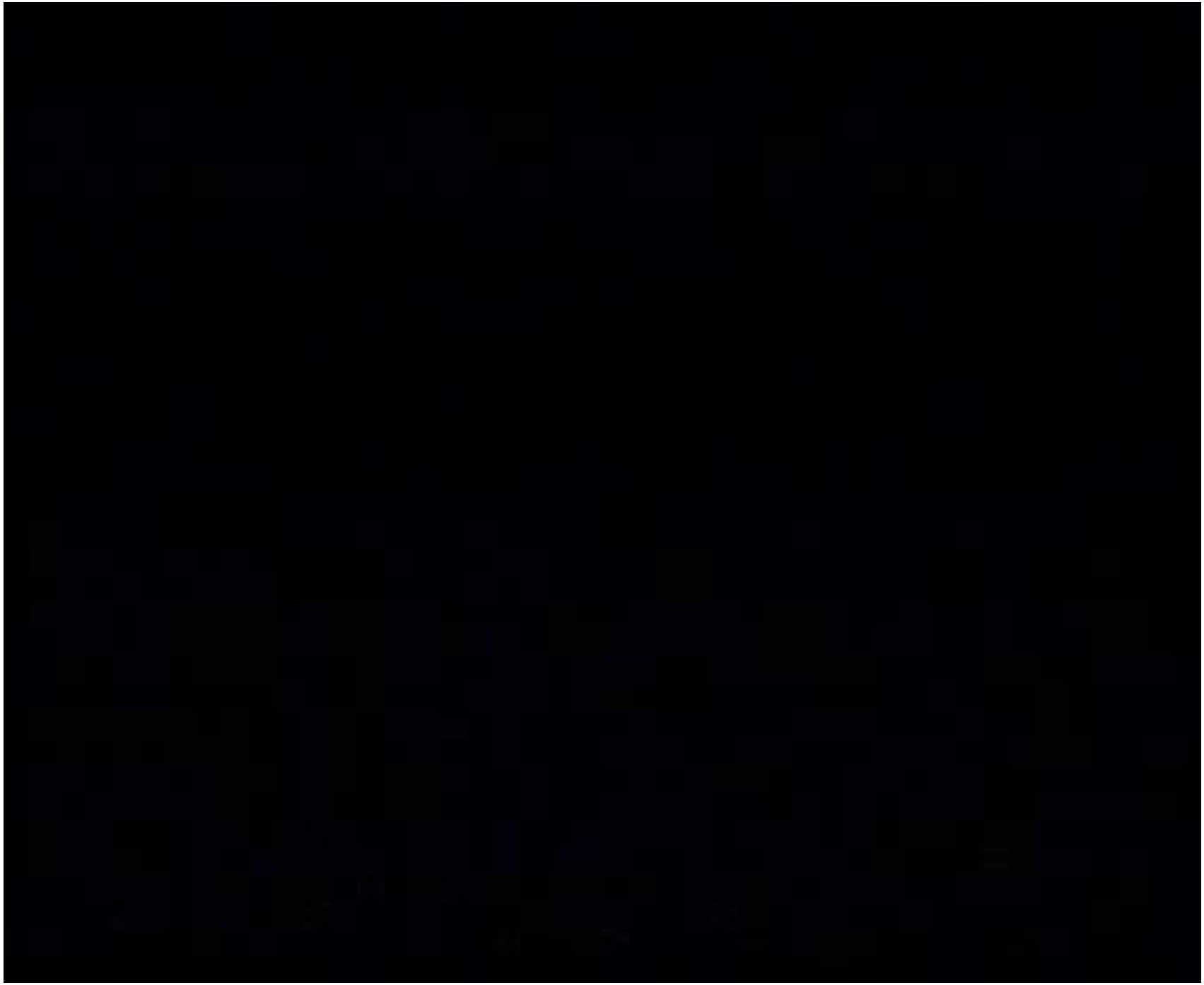
Welcome!

This conference on Analytical Ultracentrifugation is dedicated to Nobel Laureate The Svedberg (biography) at the 125th anniversary of his birth on August 30, 1884. The Svedberg is the father of the analytical ultracentrifuge, an invention that allowed the determination of particle size distributions of colloids, the determination of molecular weights of macromolecules and the proof that macromolecules exist. The ultracentrifuge helped to put The on his way to the Nobel Prize in Chemistry in 1926 for his work on disperse systems. The pioneering work of Svedberg and his colleagues on colloids and macromolecular compounds laid the foundations for far-reaching progress in molecular biology, macromolecular chemistry and biochemistry as well as colloid science. The envisaged topics of this special conference try to follow The Svedberg's broad range of scientific interests related to the Analytical Ultracentrifuge.

The Svedberg

Molecular weight: analytical ultracentrifugation


LIGHT SOURCE
CELL
SLIT, LENSES etc.
DETECTOR
PC



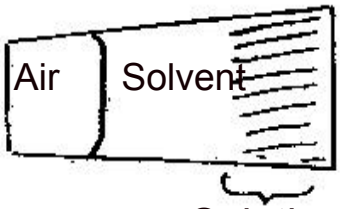
1. *Molecular weight and molecular weight distribution analysis*

2. *Conformation and flexibility analysis*
   - *general (rods, spheres, coils etc)*
   - *polymer flexibility*
   - *protein conformation: ellipsoids and bead models*

Analytical ultracentrifugaton:

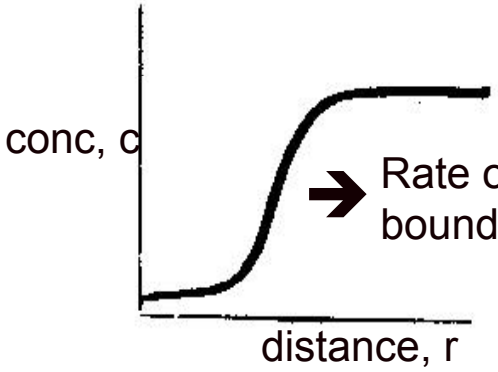
Sedimentation Velocity

Centrifugal force ➔



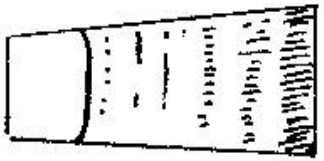
Top view, sector of centrifuge cell

Solution

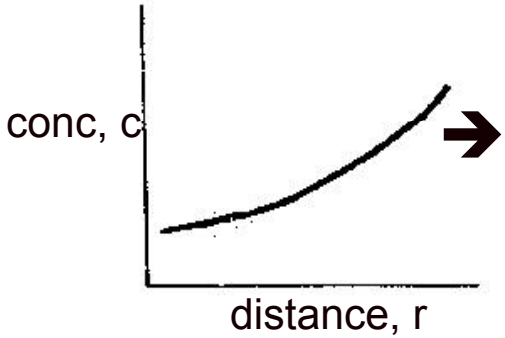


➔ Rate of movement of boundary → sed. coeff

Sedimentation Equilibrium



Centrifugal force ➔
➔Diffusion

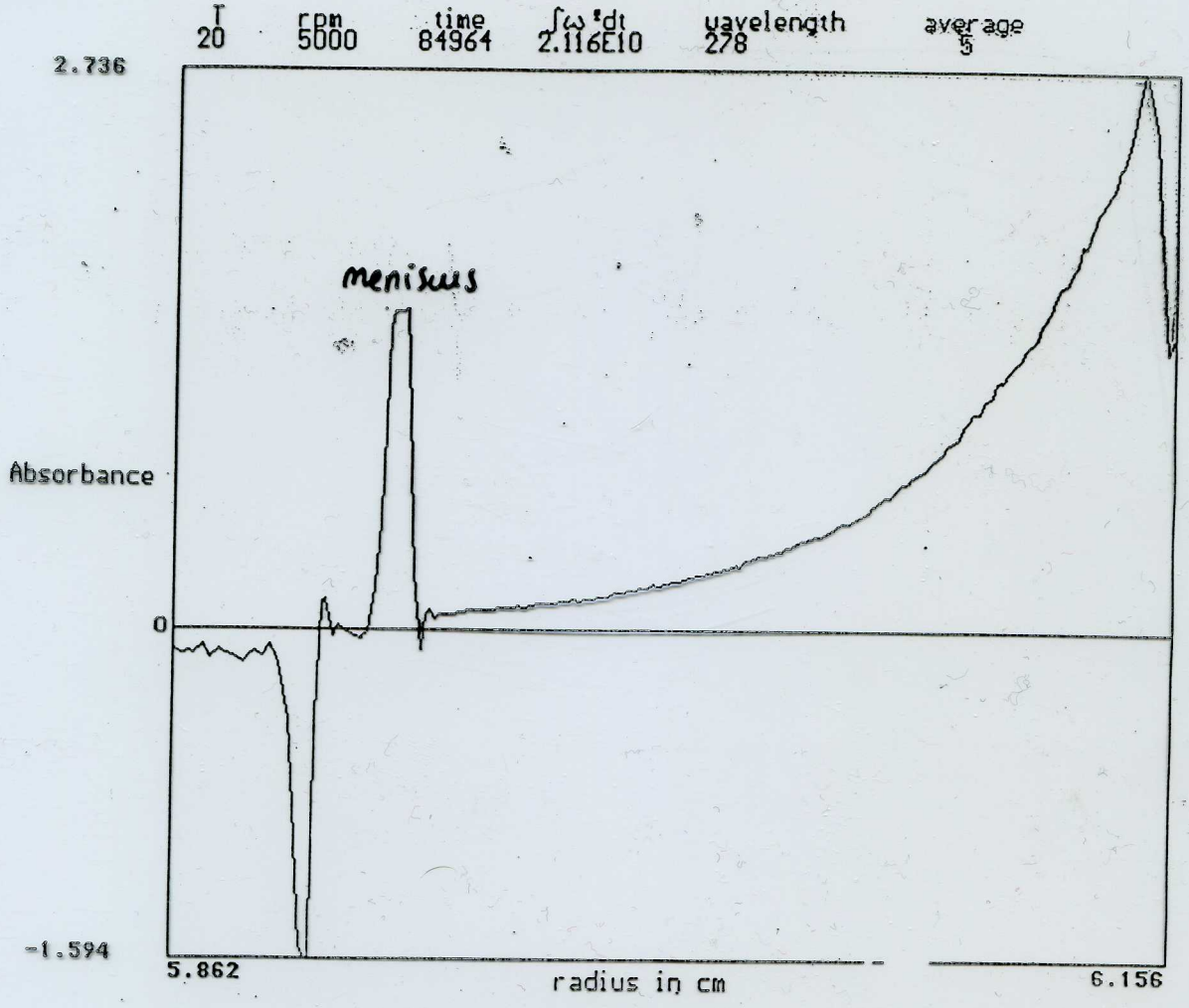


➔ STEADY STATE PATTERN

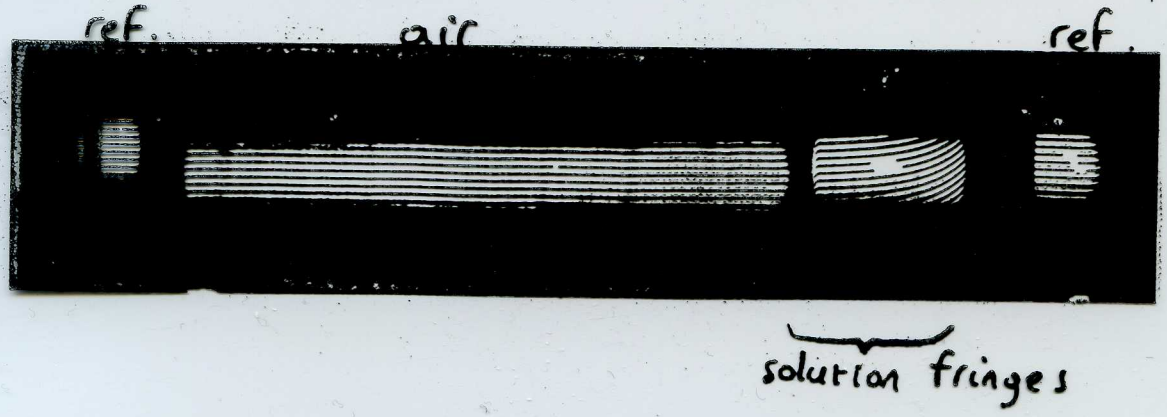
FUNCTION ONLY OF MOL. WEIGHT PARAMETERS

OPTICAL RECORDS FROM SEDIMENTATION EQUILIBRIUM

Absorption (IgM) $M_w \sim 1.0 \times 10^6$



Interference (reduced mucin) $M_w \sim 500000$



SEDIMENTATION EQUILIBRIUM TRACE :

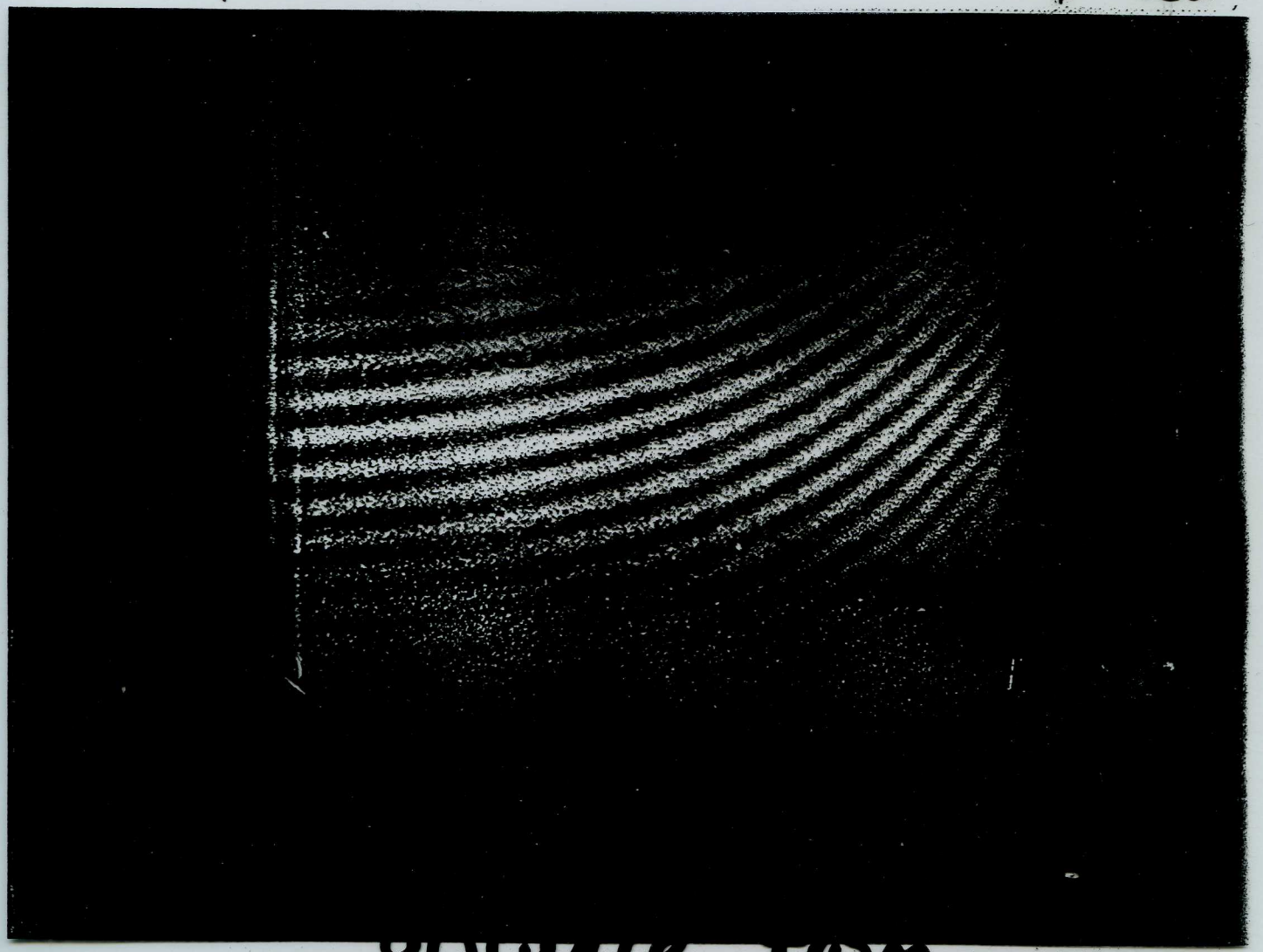
Interference Optics - Ovalbumin

$M_N = 45000$

centrifugal field →

m (meniscus) ↓

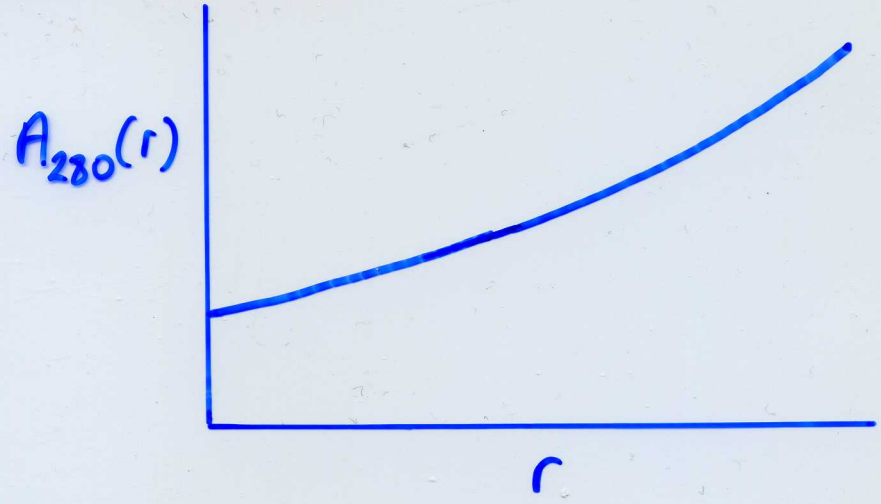
b (base cell) ↓



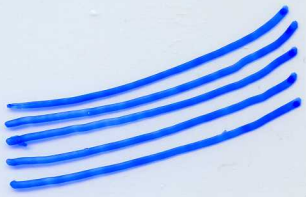
distance from
rotor centre

∴ To Summarize both uv-absorption and interference optics provide a record of the concentration distribution of sedimentation equilibrium in the ultracentrifuge cell

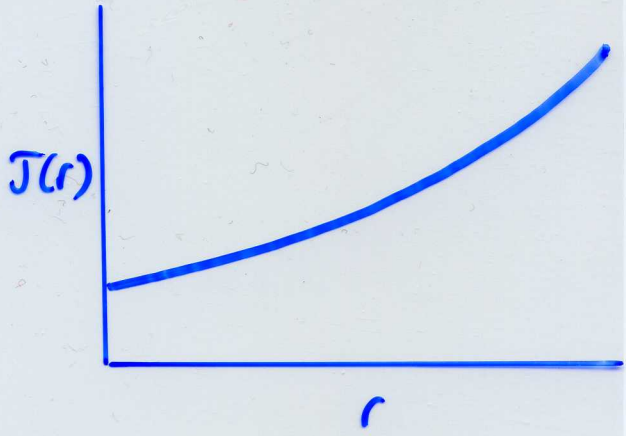
① UV ABSORPTION



② INTERFERENCE



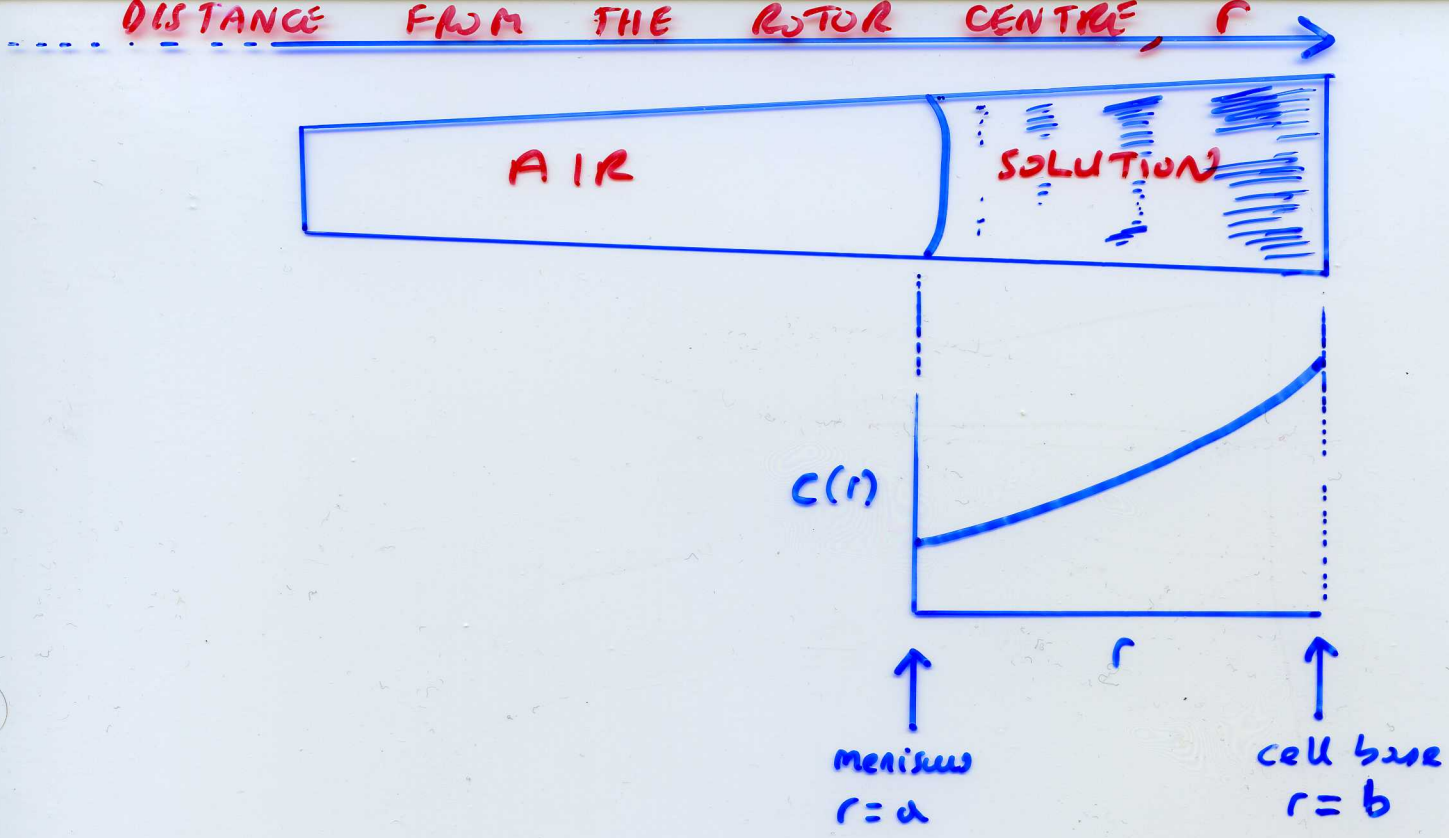
Fourier transform + allowance for meniscus concentration →



IN BOTH CASES THE RECORD IS A DIRECT RECORD OF CONCENTRATION

$A(r) \propto c(r)$

$J(r) \propto c(r)$



In working out molecular weights we can either use concentrations in the centrifuge cell, $c(r)$, directly (g/ml) or use absorbances $A(r)$ or fringe displacements $J(r)$

At <u>sedimentation Equilibrium</u> sedimentation & diffusion forces are = and opposite

$$\omega^2 r M (1 - \bar{v}\rho) = \frac{RT}{c(r)} \frac{dc(r)}{dr}$$

(from sedimentation) (from diffusion)

$$\therefore \frac{1}{c(r)} \frac{dc(r)}{dr} = \frac{\omega^2 r M (1 - \bar{v}\rho)}{RT}$$

N.B. For heterogeneous / non-ideal systems M should be $M_{w,app}$

THE IMPORTANT EQUATIONS FOR SEDIMENTATION EQM.

Fundamental equation : $$\frac{1}{c(r)}\frac{dc(r)}{dr} = \frac{\omega^2 r M_{w,app}(1-\bar{v}\rho)}{RT}$$

+ its integrated form : $$c(r) = c(a)\, e^{\omega^2 M_{w,app}(1-\bar{v}\rho)(r^2-a^2)/2RT}$$

These can be manipulated in a number of ways

① Logarithmic form over the whole distribution (from $r=a$ to $r=b$)

$$M_{w,app} = \left[\ln \frac{c(b)}{c(a)}\right] \cdot \frac{2RT}{\omega^2(1-\bar{v}\rho)(b^2-a^2)}$$

(n.b. at low concentration $M_{w,app} \simeq M_w$)

② Integral form over the whole distribution

$$M_{w,app} = \left[\frac{c(b)-c(a)}{c^0}\right] \frac{2RT}{\omega^2(1-\bar{v}\rho)(b^2-a^2)}$$

c^0 : initial concentration loaded into the centrifuge cell

③ "Local" or point average molecular weights

$$M_{w,app}(r) = \frac{d\ln c(r)}{dr^2} \cdot \frac{2RT}{(1-\bar{v}\rho)\omega^2}$$

④ M^* form

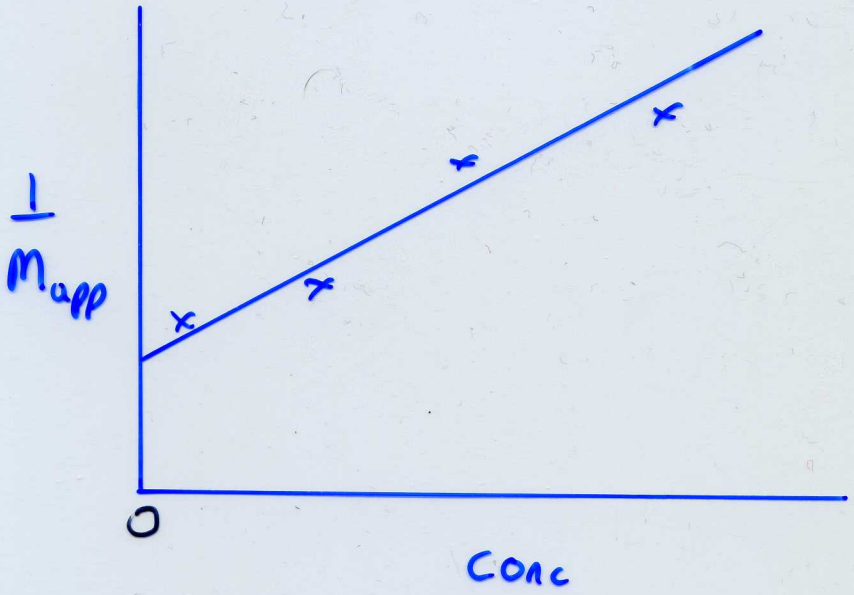
$$M^*(r) = \frac{c(r) - c(a)}{\left[\frac{(1-\bar{v}\rho)\omega^2}{2RT}\right] \cdot \left[c(a)(r^2-a^2) + 2\int_a^r r\{c(r)-c(a)\}dr\right]}$$

and $M_{w,app} = M^*(r \to b)$

THERMODYNAMIC NON-IDEALITY

Values for M so obtained (at a finite concentration c) are only apparent values (M_{app}) because of non-ideality [although in practise, for proteins $M_{app} \approx M$ especially for concentrations $\lesssim 1.0$ mg/ml]

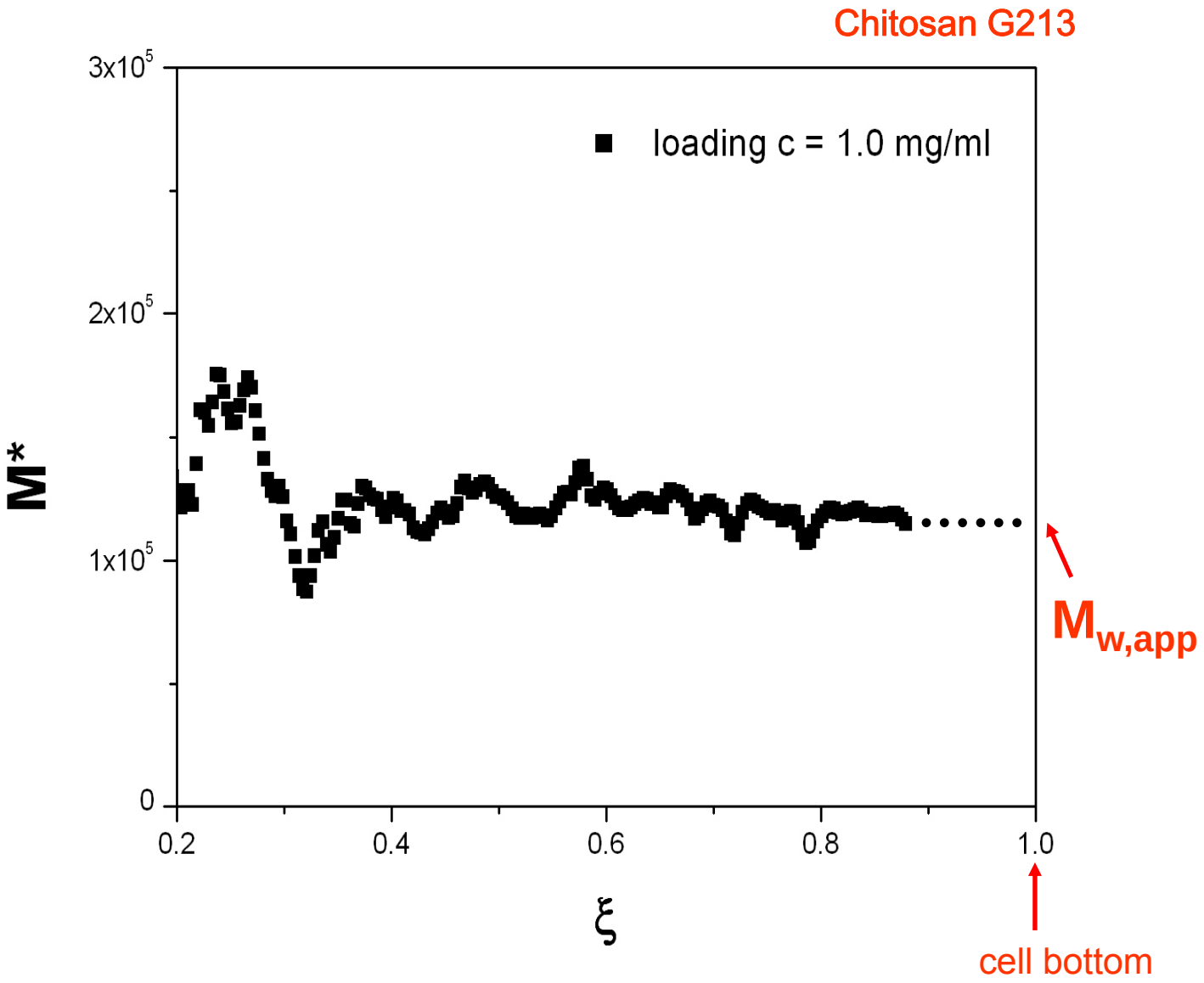
For glycoconjugates & polysaccharides, an extrapolation MAY be necessary



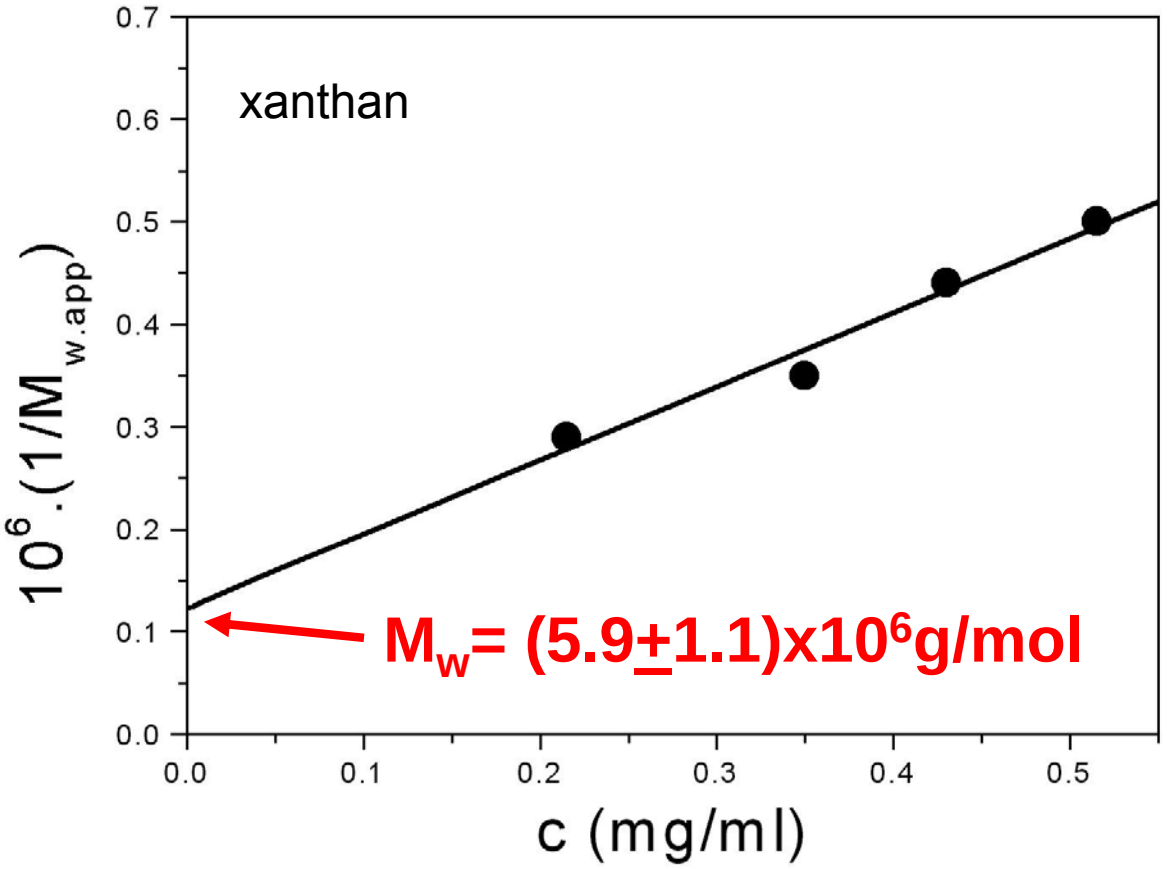
$$\frac{1}{M_{app}} = \frac{1}{M}(1 + 2BMc)$$

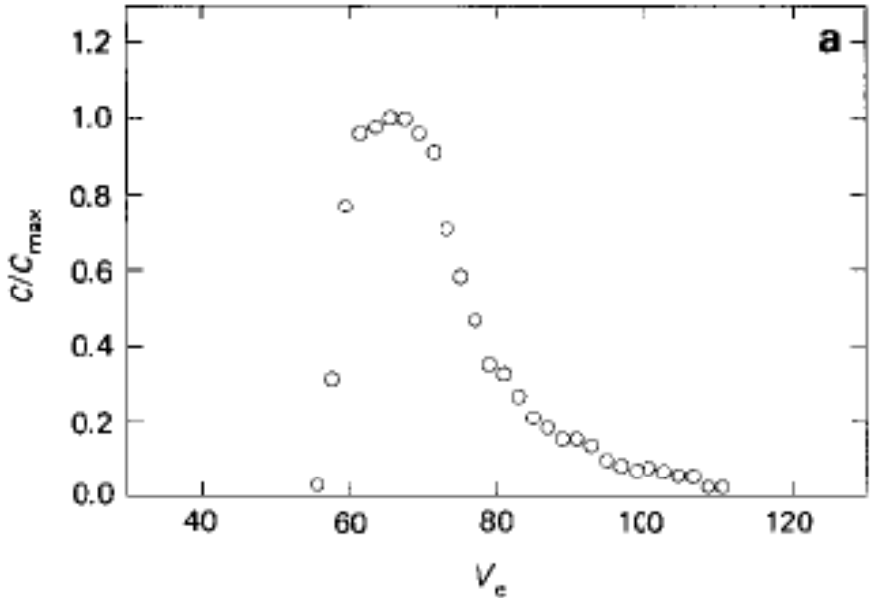
B: 2nd virial coefficient

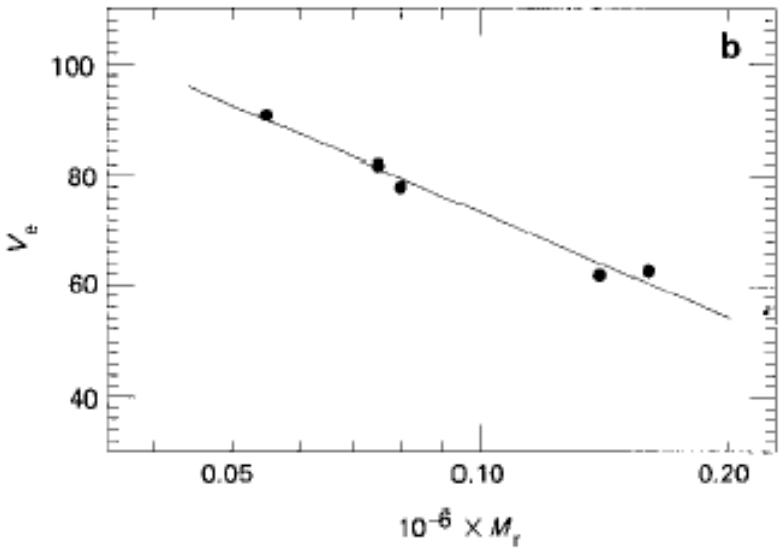
Extraction of $M_{w,app}$ from sedimentation equilibrium and "MSTAR" analysis

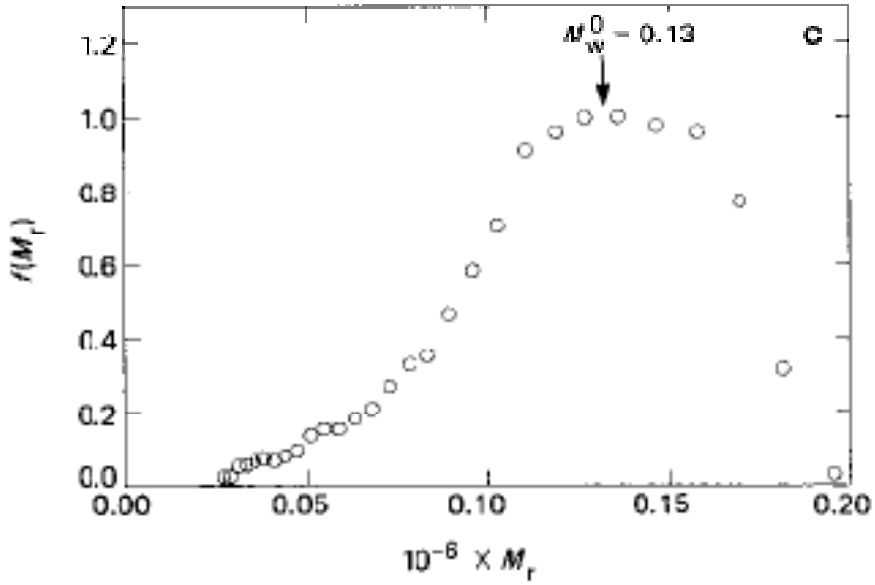


Extraction of $M_{w,app}$ from sedimentation equilibrium and "MSTAR" analysis









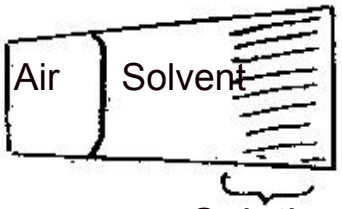
SEC - sedimentation equilibrium

mol. wt distribution: alginate

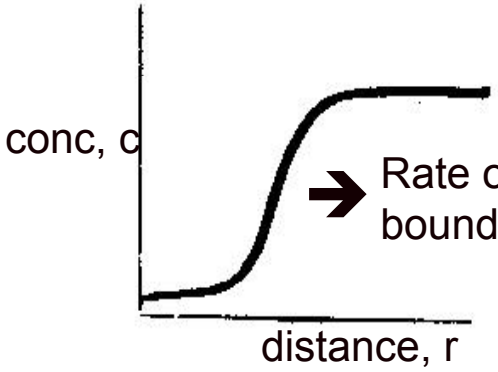
Ball A, Harding SE & Mitchell J,
Int. J. Biol. Macromol., 1988

Sedimentation Velocity

Centrifugal force ➔

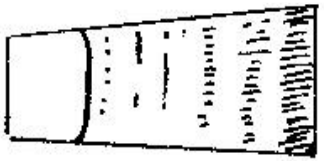


Top view, sector of centrifuge cell

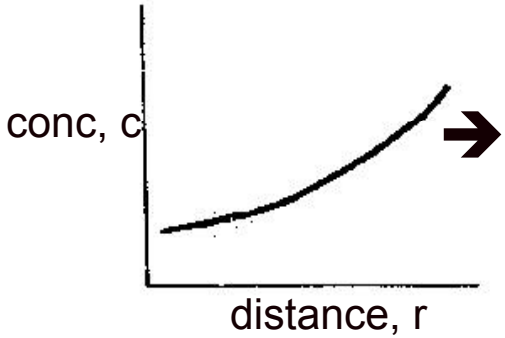


➔ Rate of movement of boundary → sed. coeff

Sedimentation Equilibrium



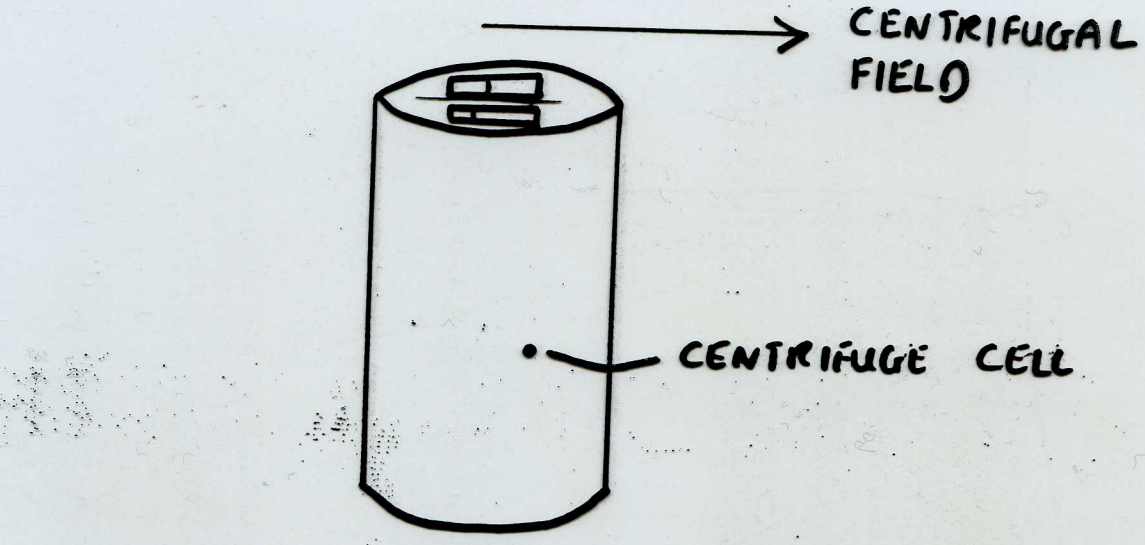
Centrifugal force ➔
➔Diffusion

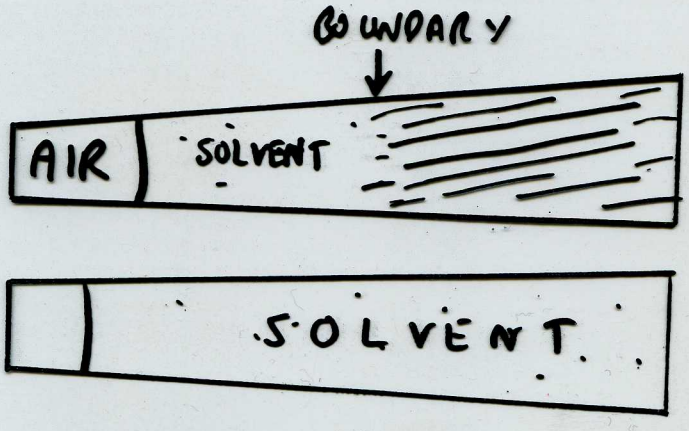


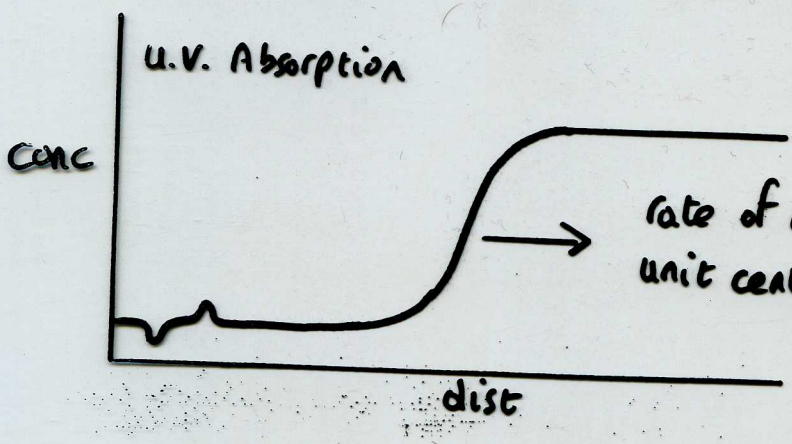
➔ STEADY STATE PATTERN

FUNCTION ONLY OF MOL. WEIGHT PARAMETERS

SEDIMENTATION VELOCITY

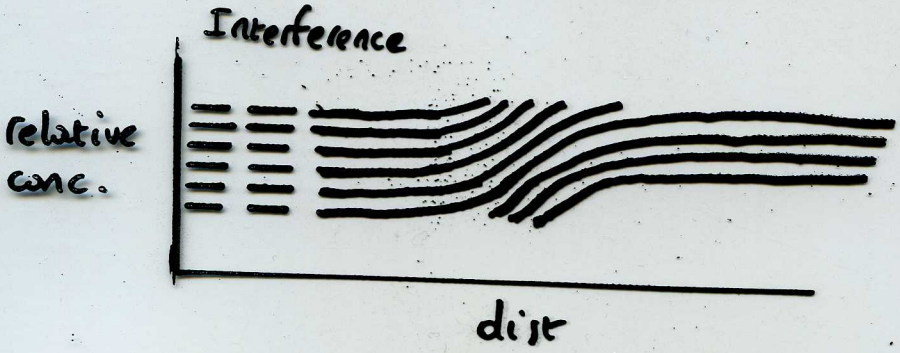






rate of movement / unit centrifugal field → SEDIMENTATION COEFFICIENT

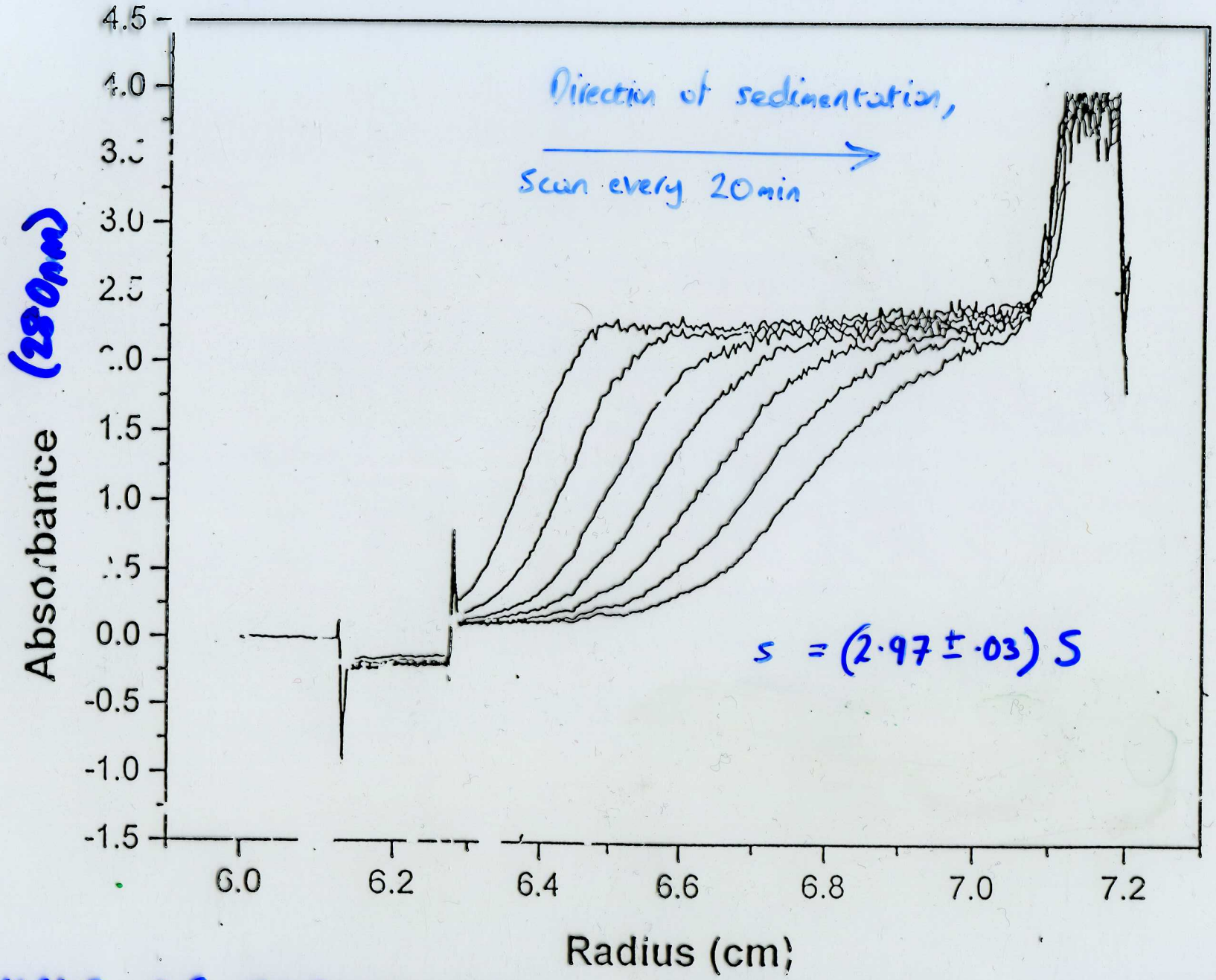
$S^{0}_{20,w}$



UNIT: SVEDBERGS

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

β-LACTOGLOBULIN, 25.0°C, 50000 rpm

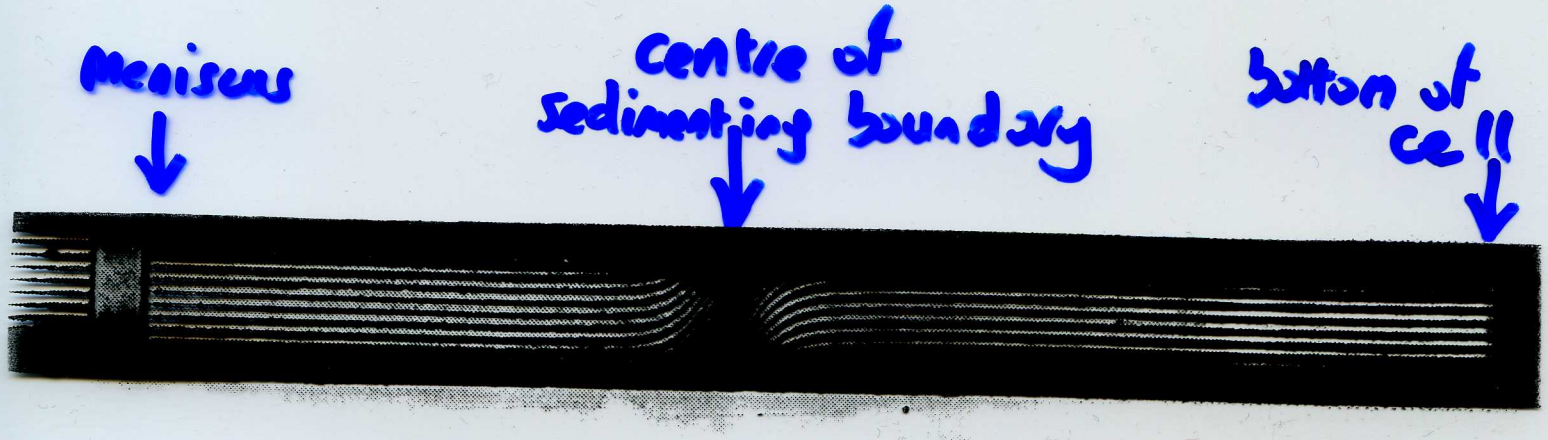


EXAMPLE OF SEDIMENTATION VELOCITY USING ABSORPTION OF UV

Interference Optics:

Tomato Bushy Stunt Virus

11000 rpm 20.0°C $s = (130 \pm 2)\ S$



Direction of Sedimentation

EXAMPLE OF SEDIMENTATION VELOCITY USING REFRACTOMETRIC (INTERFERENCE) OPTICS

Sedimentation velocity

sedimentation coefficient, s

$$= \frac{\text{velocity}}{\text{centrifugal field}}$$

$$= \frac{v}{\omega^2 r}$$

ω = angular velocity of rotor (radians / sec)

r = distance of particle from centre of rotor

measured from rate of movement of boundary with time

s values often corrected to standard conditions for comparison purposes (20°C in water)

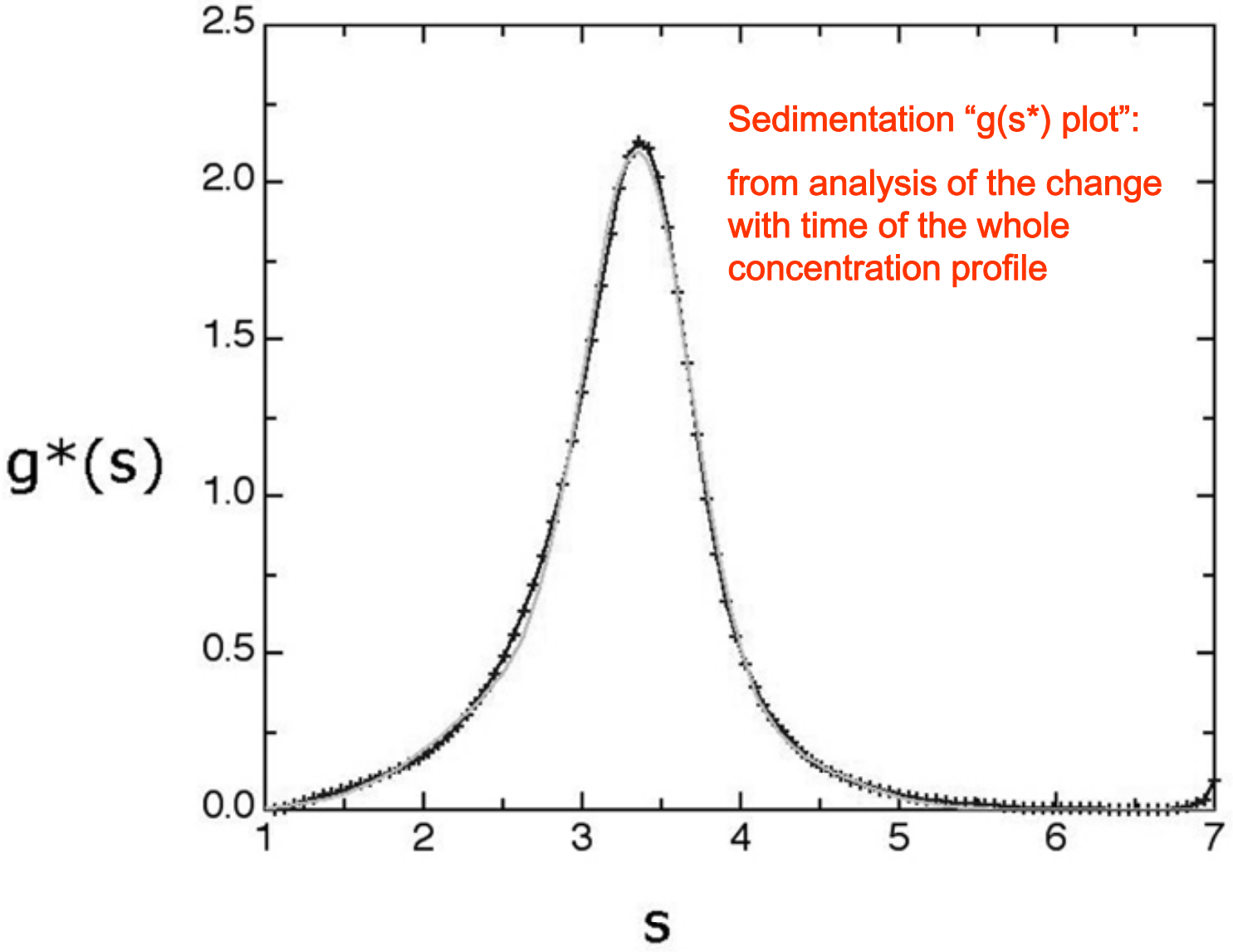
Lysosyme : $s = 1.91 \times 10^{-13}$ sec

Bovine serum albumin, $s = 5.01 \times 10^{-13}$ sec

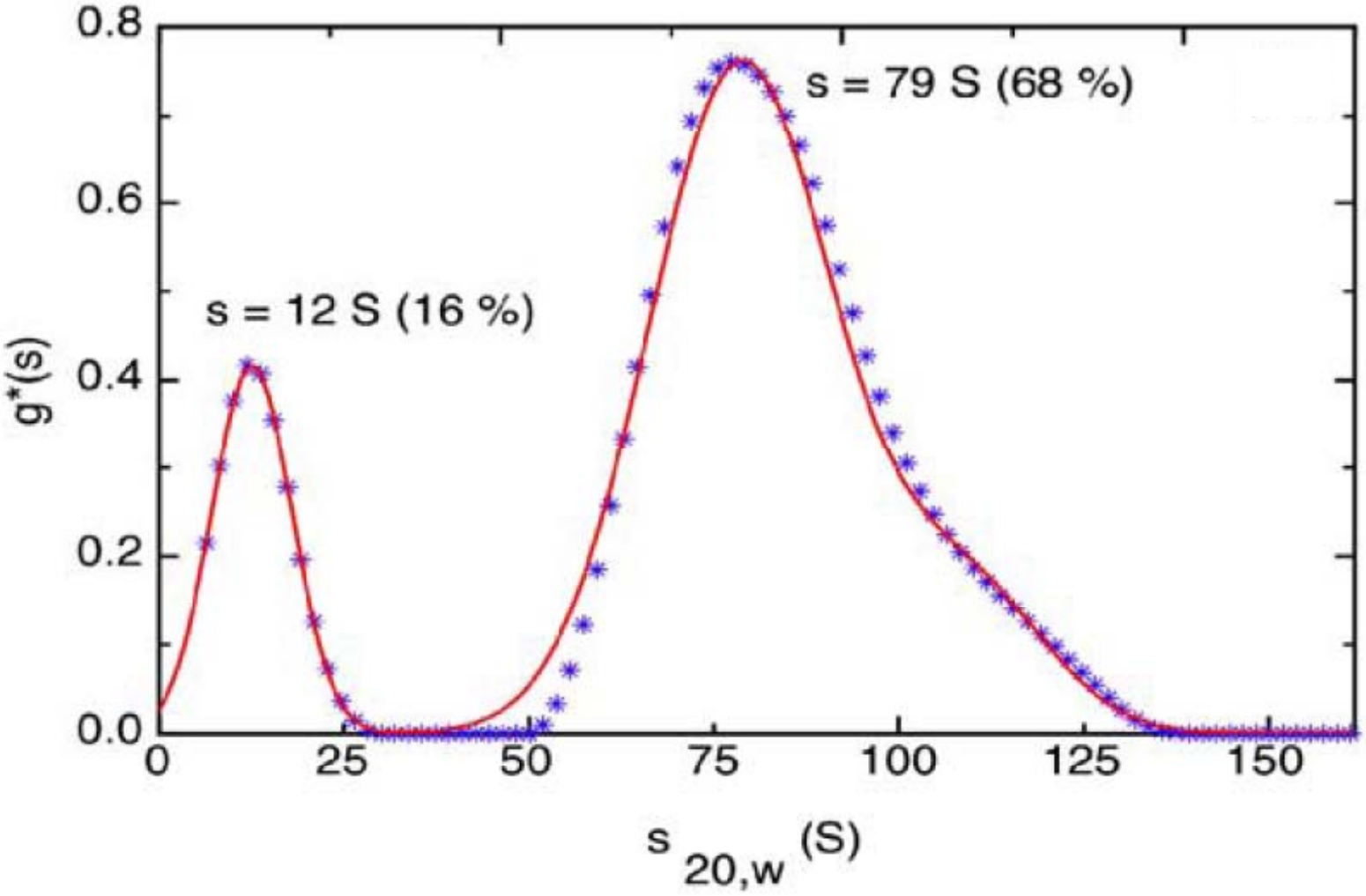
Fibrinogen $s = 7.9 \times 10^{-13}$ sec

common unit : The 'Svedberg' $S = 10^{-13}$ sec

Guar, 0.75 mg/ml

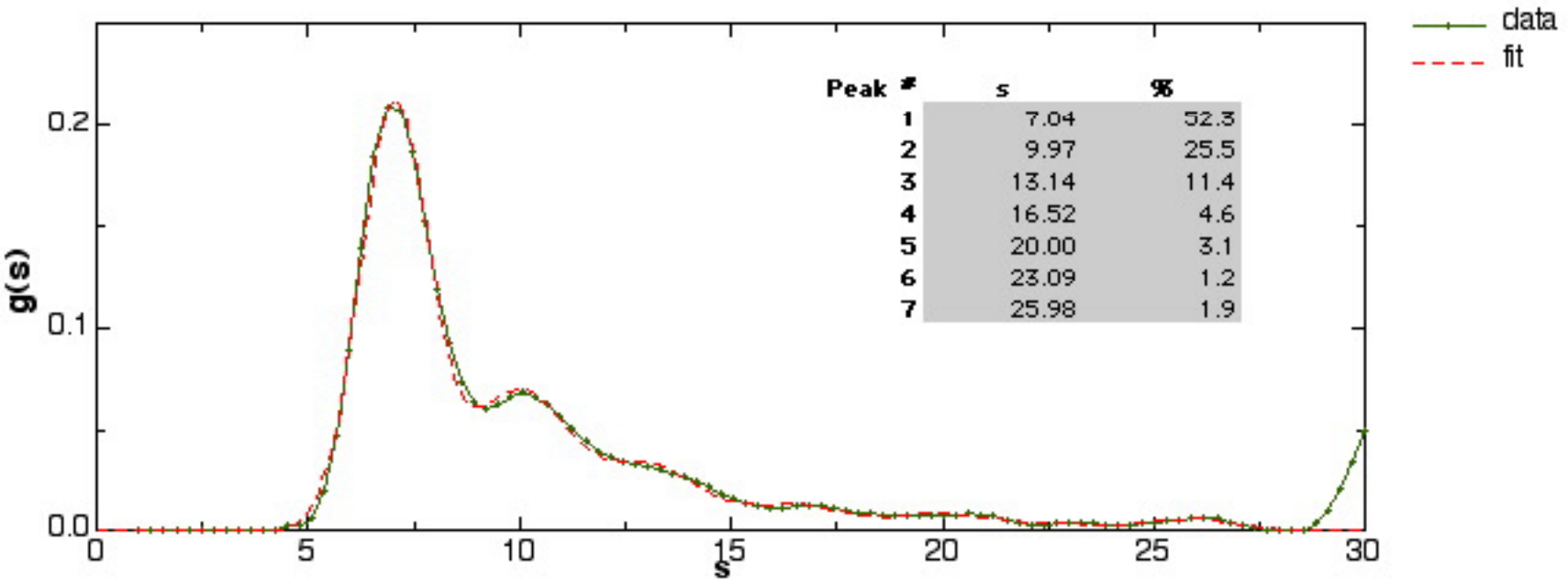


Sedimentation velocity g(s) plot: starch*



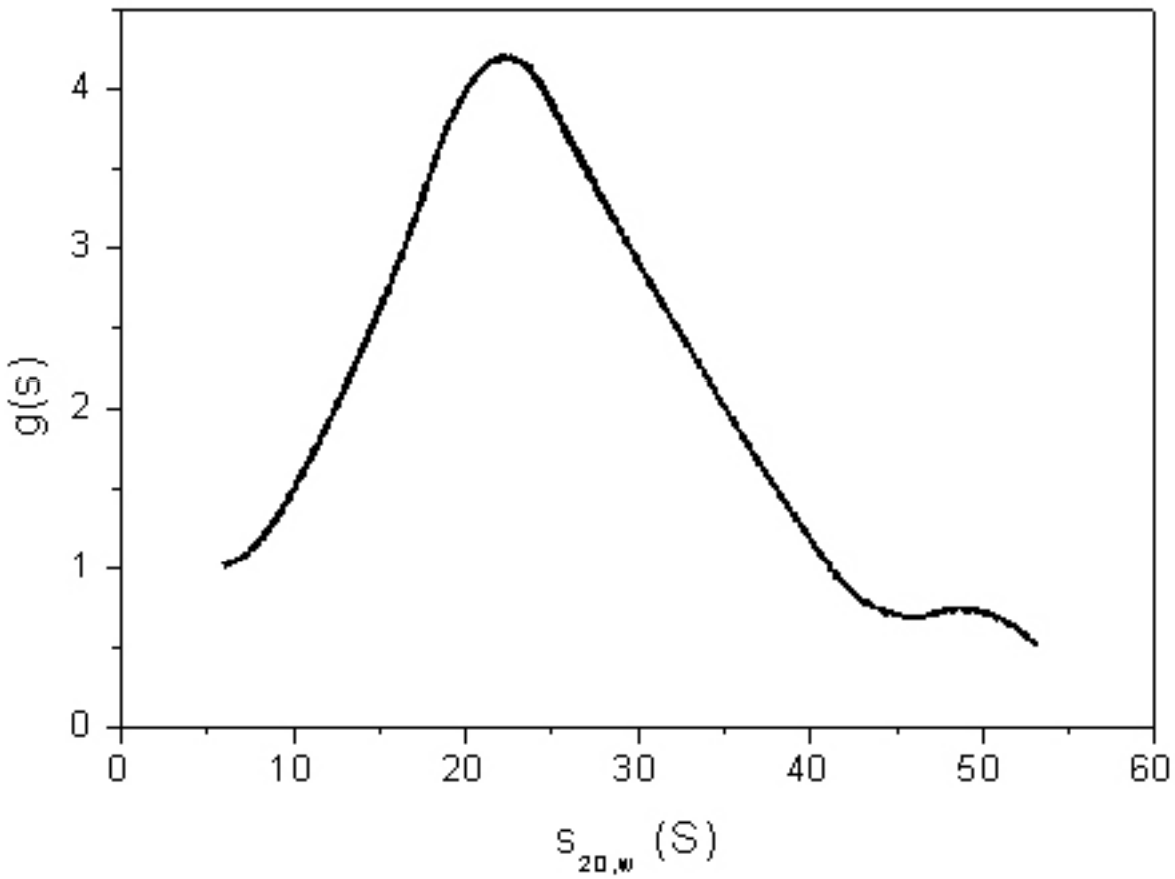
Tester R, Patel T, Harding, S. *Carbohydrate Research,* 2006

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



Peak #	s	%
1	7.04	52.3
2	9.97	25.5
3	13.14	11.4
4	16.52	4.6
5	20.00	3.1
6	23.09	1.2
7	25.98	1.9

Converting a sedimentation coefficient distribution to a molecular weight distribution

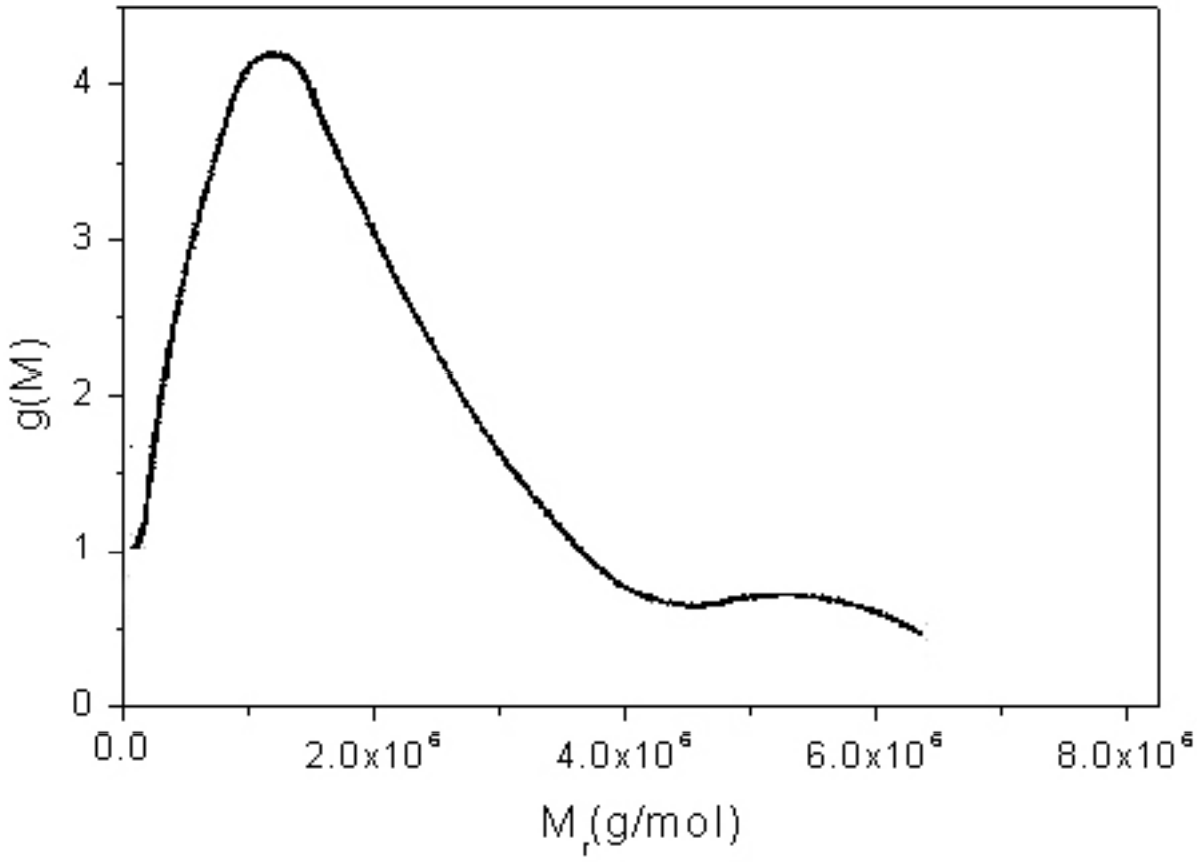


$s_{20,w} \sim M^b$

Harding, S. *Adv. Carb. Chem. Biochem,* 1989

Converting a sedimentation coefficient distribution to a molecular weight distribution

Molecular weight distribution – no column or membrane needed

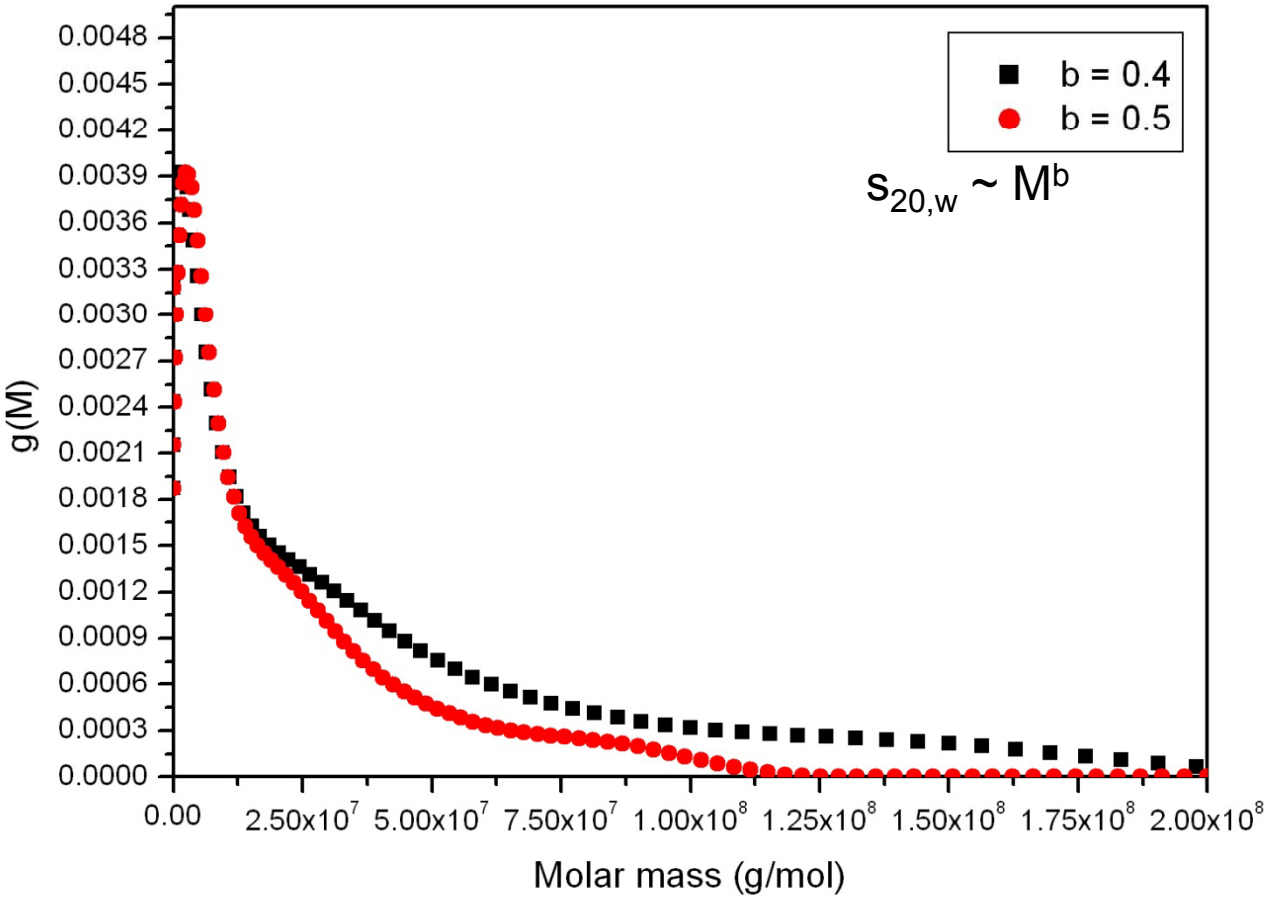


$s_{20,w} \sim M^b$ (b=0.5)

Harding, S. *Adv. Carb. Chem. Biochem,* 1989

Converting a sedimentation coefficient distribution to a molecular weight distribution

Glycoconjugate vaccine – too large for SEC-MALLs analysis

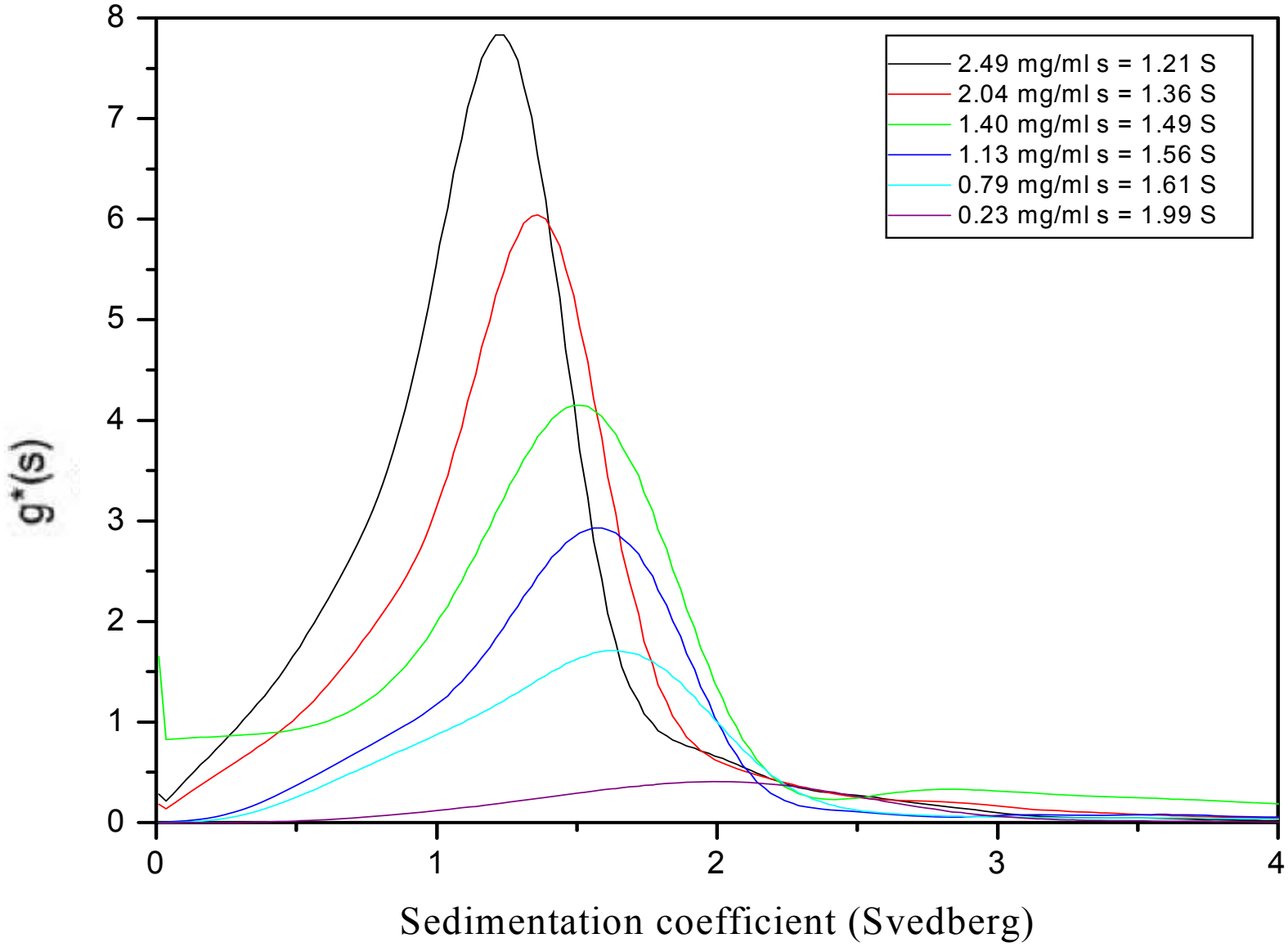


Harding, S., Abdelhameed, A., Morris, G. (2010)

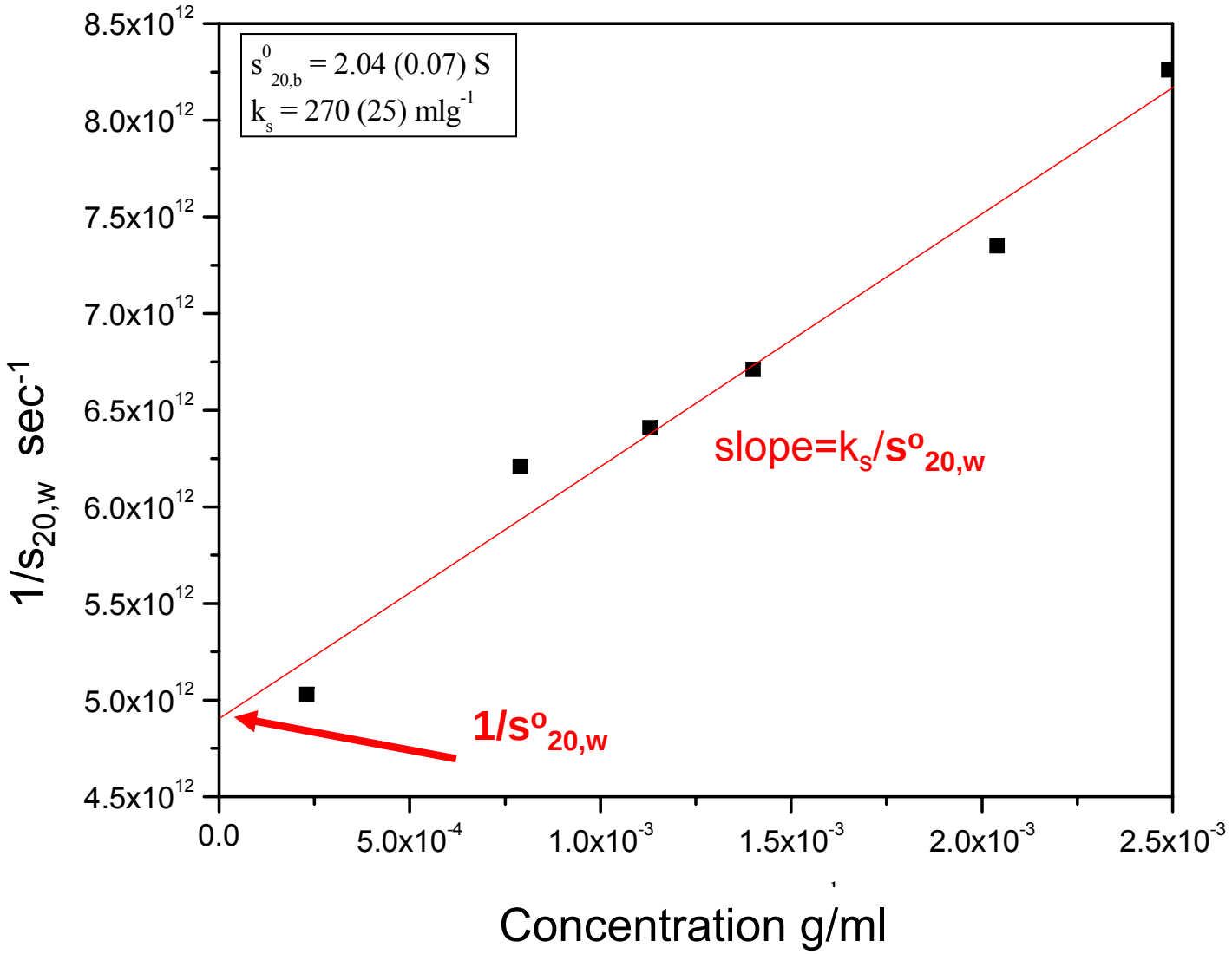
1. *Molecular weight and molecular weight distribution analysis*

2. *Conformation and flexibility analysis*
   - *general (rods, spheres, coils etc)*
   - *polymer flexibility*
   - *protein conformation: ellipsoids and bead models*

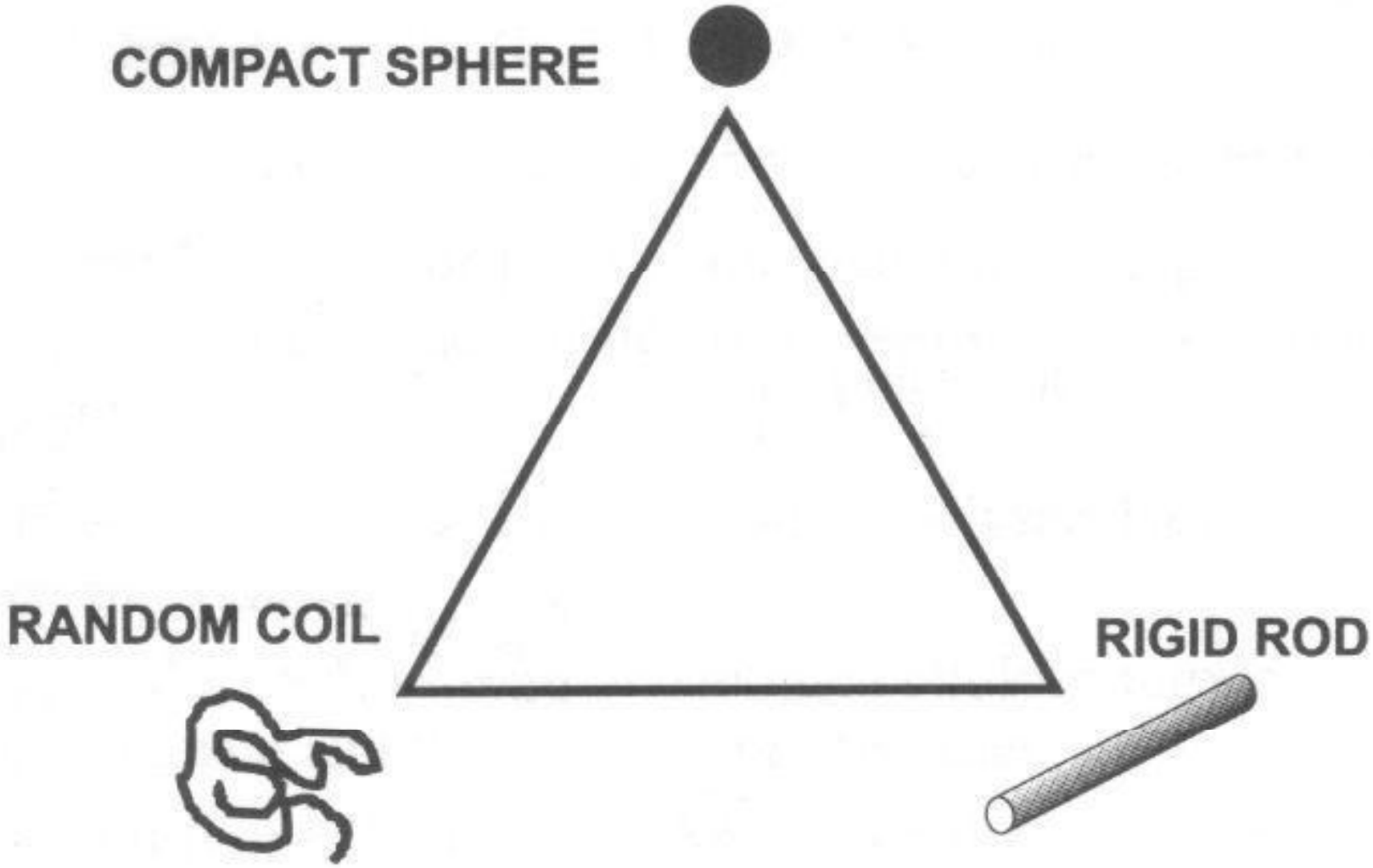
Citrus pectin

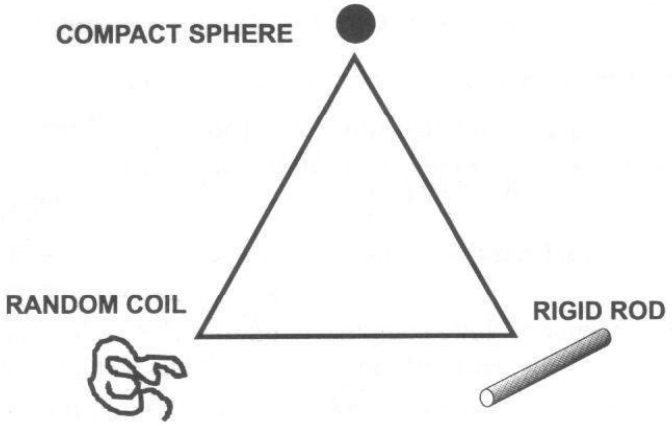


$s^{o}_{20,w}$ and k_s extraction



General conformation analysis: the Haug Triangle



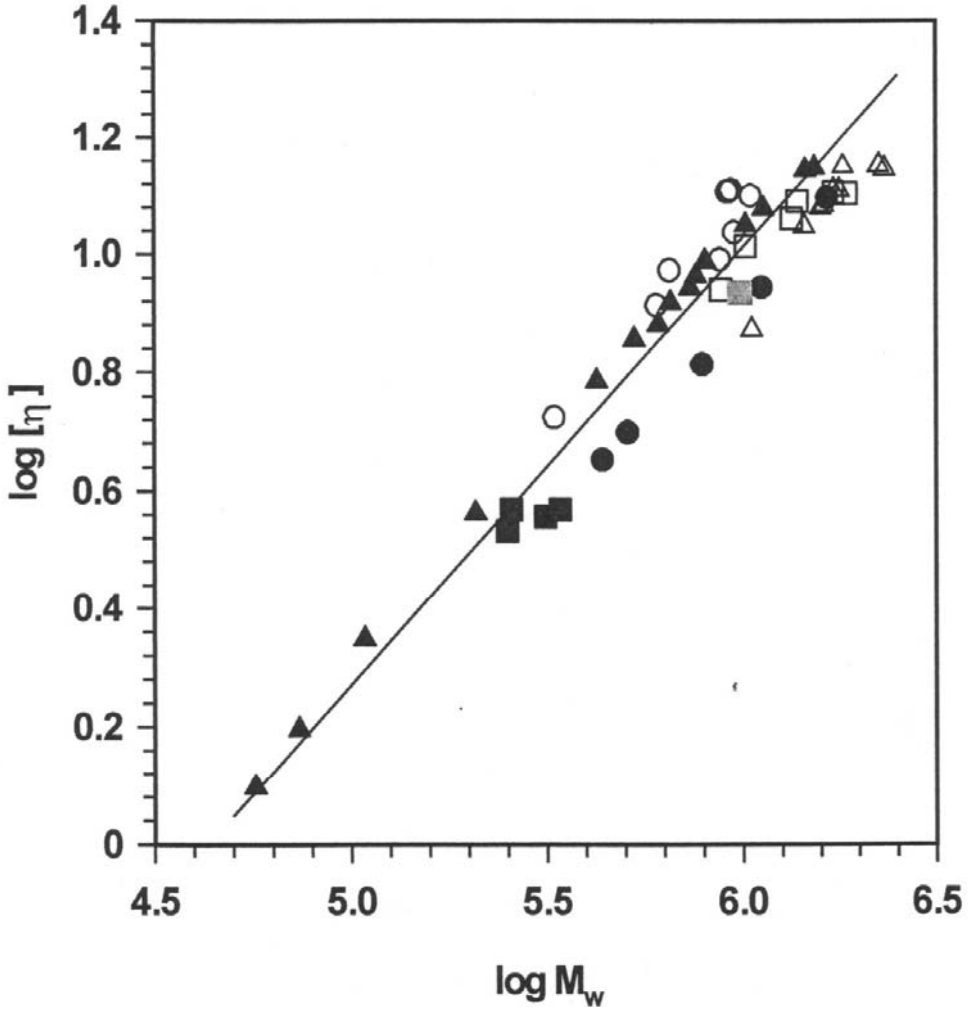


Power law, "Scaling" or "MHKS" relations:

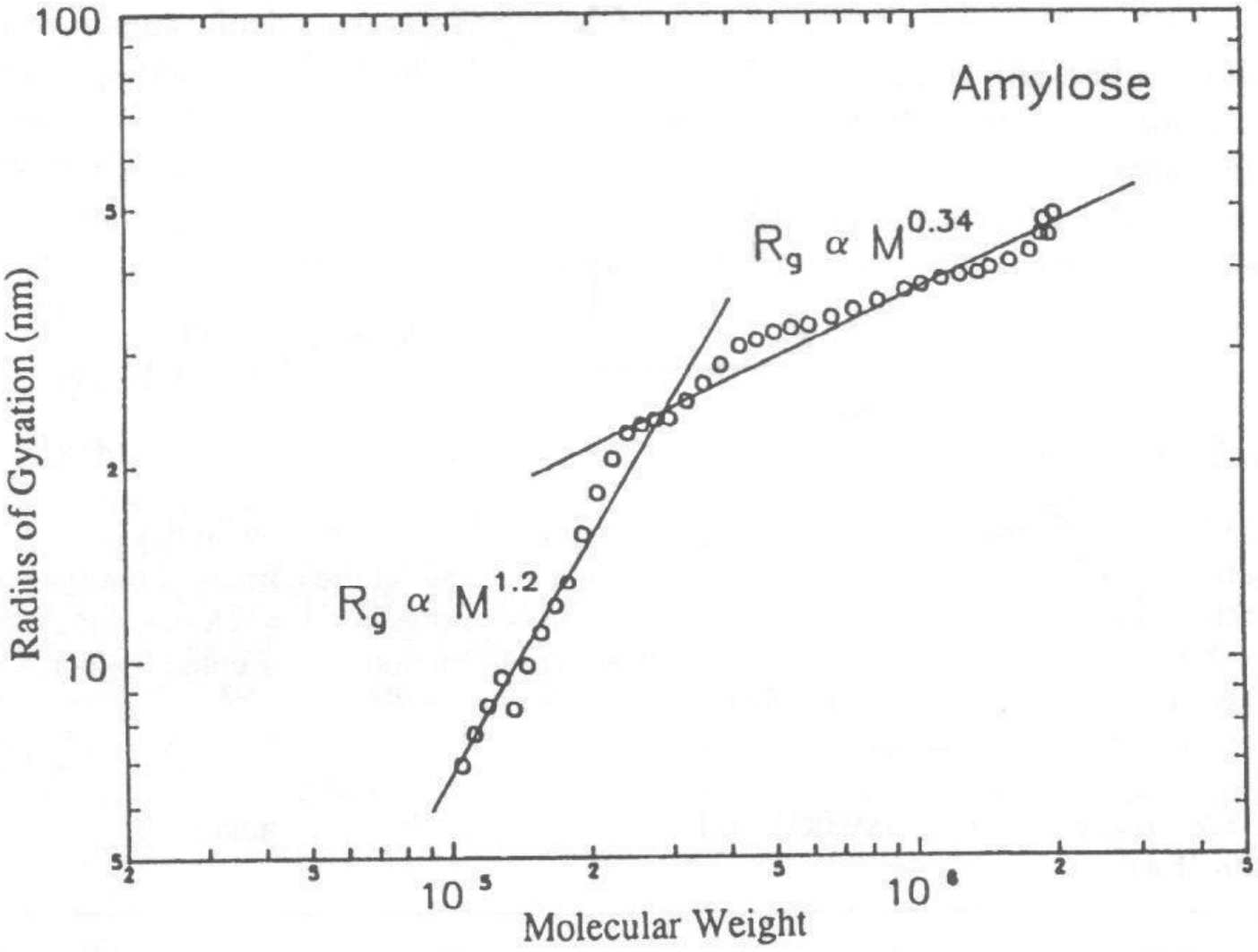
Sphere	Rod	Coil
$[\eta] \sim M^{0}$	$[\eta] \sim M^{1.8}$	$[\eta] \sim M^{0.5\text{-}0.8}$
$s^{o}_{20,w} \sim M^{0.67}$	$s^{o}_{20,w} \sim M^{0.15}$	$s^{o}_{20,w} \sim M^{0.4\text{-}0.5}$
$R_g \sim M^{0.33}$	$R_g \sim M^{1.0}$	$R_g \sim M^{0.5\text{-}0.6}$

Mark-Houwink-Kuhn-Sakurada Power law plot

Galactomannans
a=0.74±0.01



Change in Conformation



Rollings, 1992

Conformation Zoning:

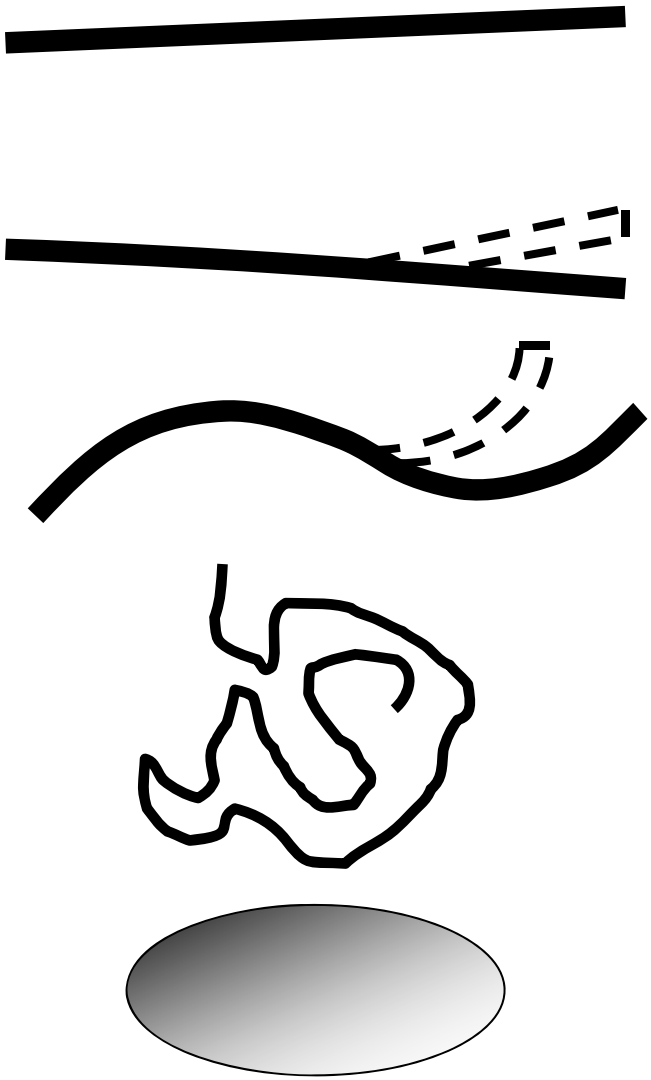
Zone A: Extra-rigid rod: schizophyllan

Zone B: Rigid Rod: xanthan

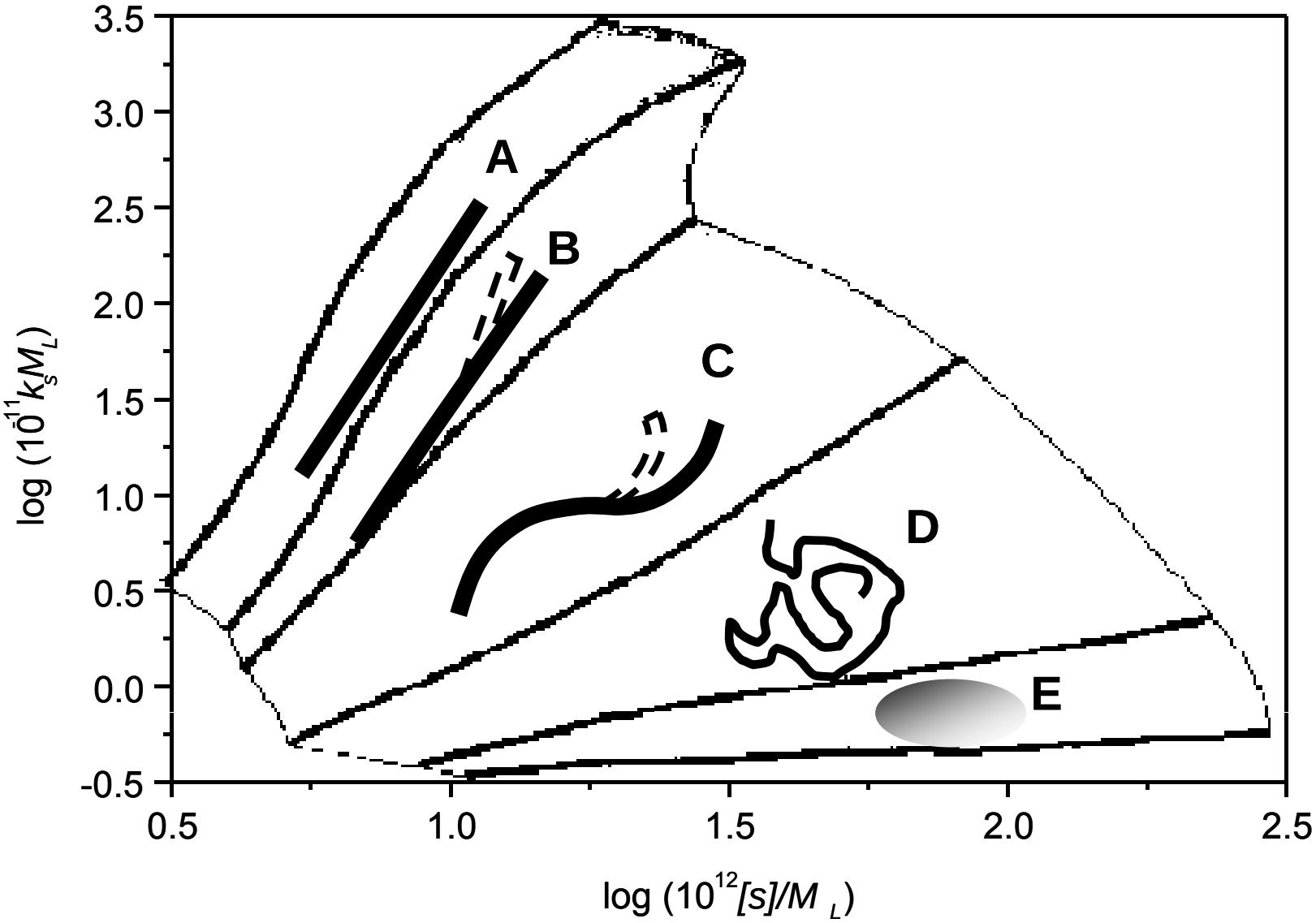
Zone C: Semi-flexible coil: pectin

Zone D: Random coil: dextran, pullulan

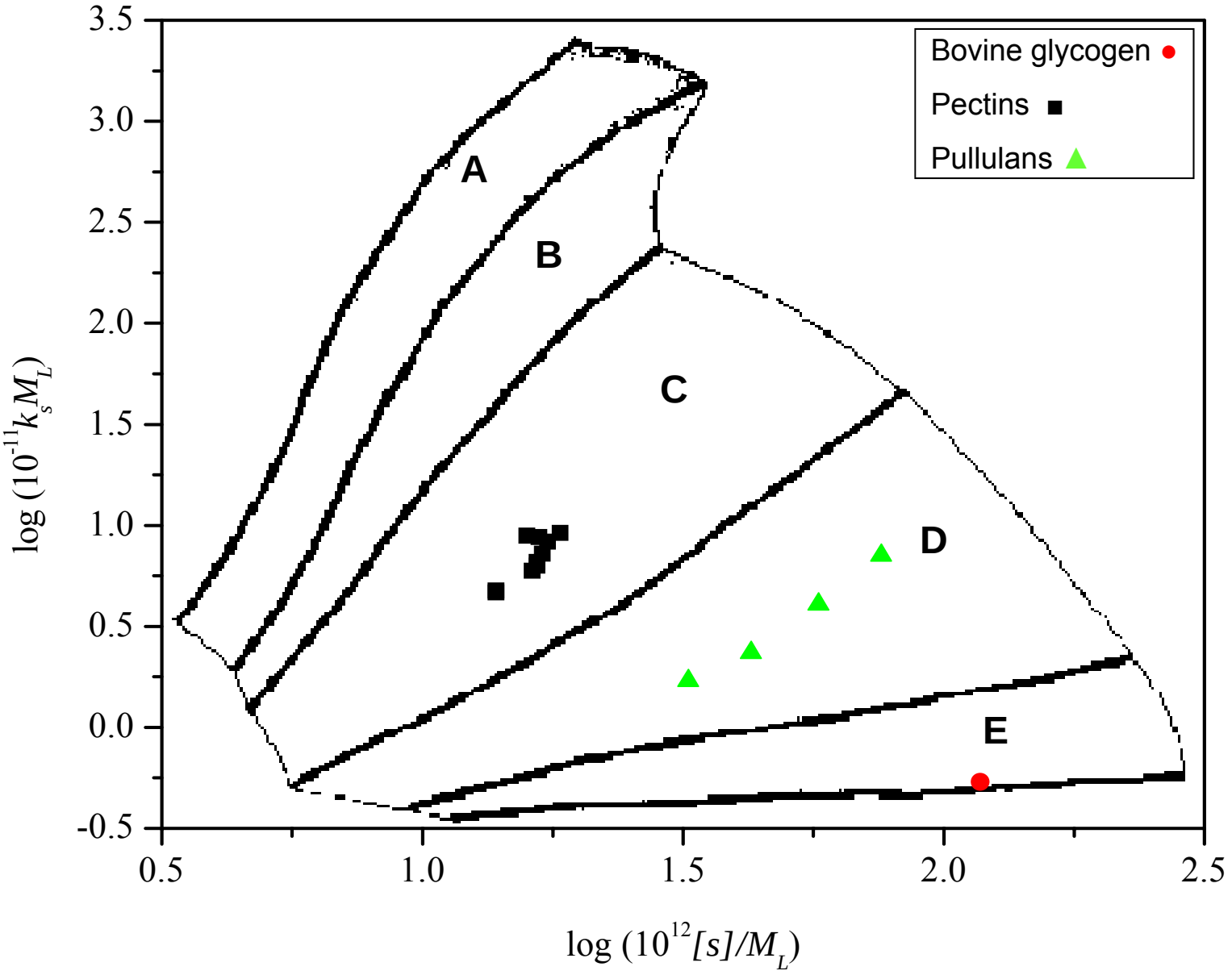
Zone E: Highly branched: amylopectin, glycogen



Conformation Zoning:

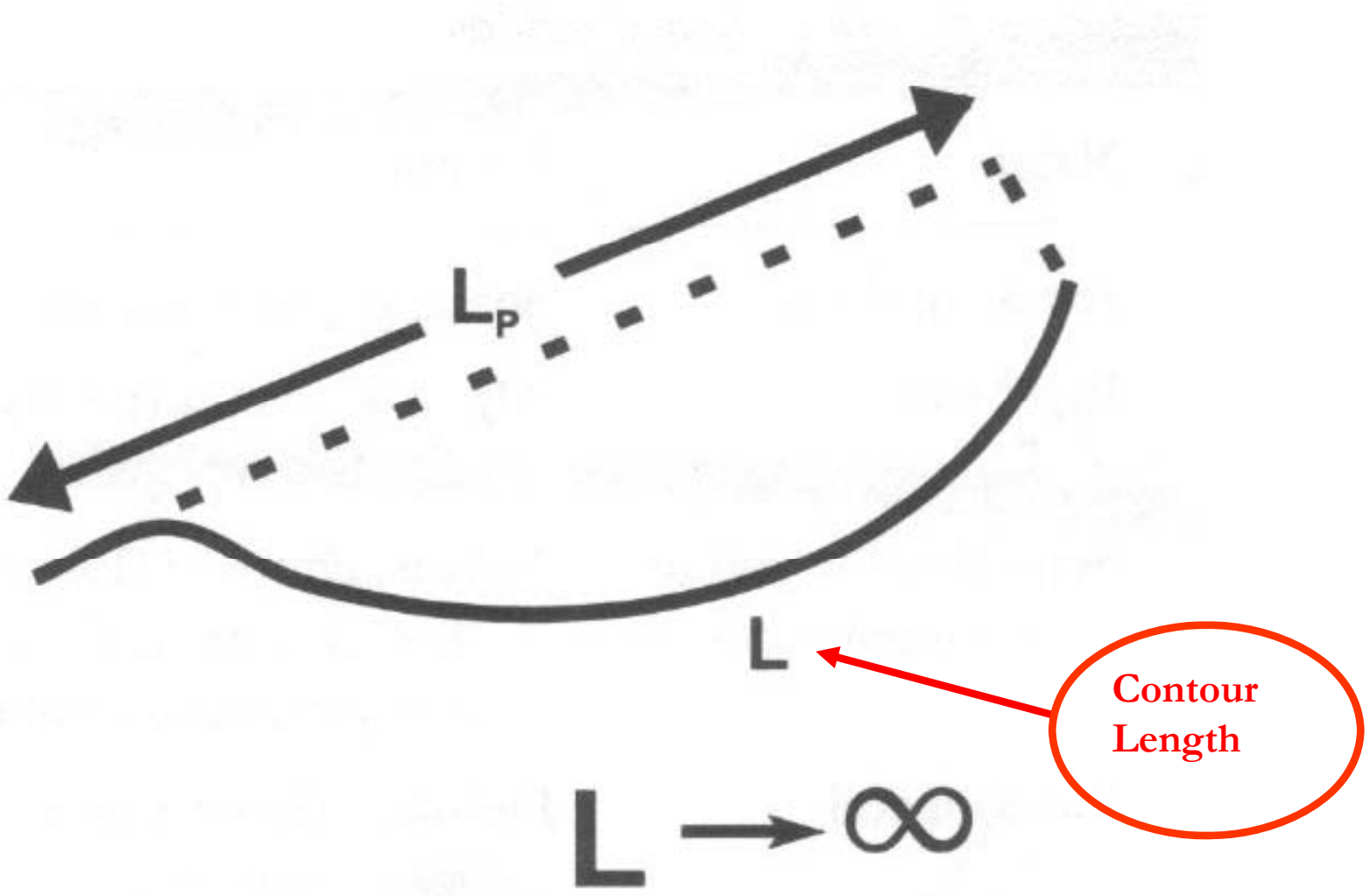


Pavlov, Rowe & Harding, *Trends in Analytical Chemistry*,1997


Bovine glycogen
Pectins
Pullulans
A
B
C
D
E
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0.5
1.0
1.5
2.0
2.5
log (10^-11 k_s M_L)
log (10^12 [s]/M_L)

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

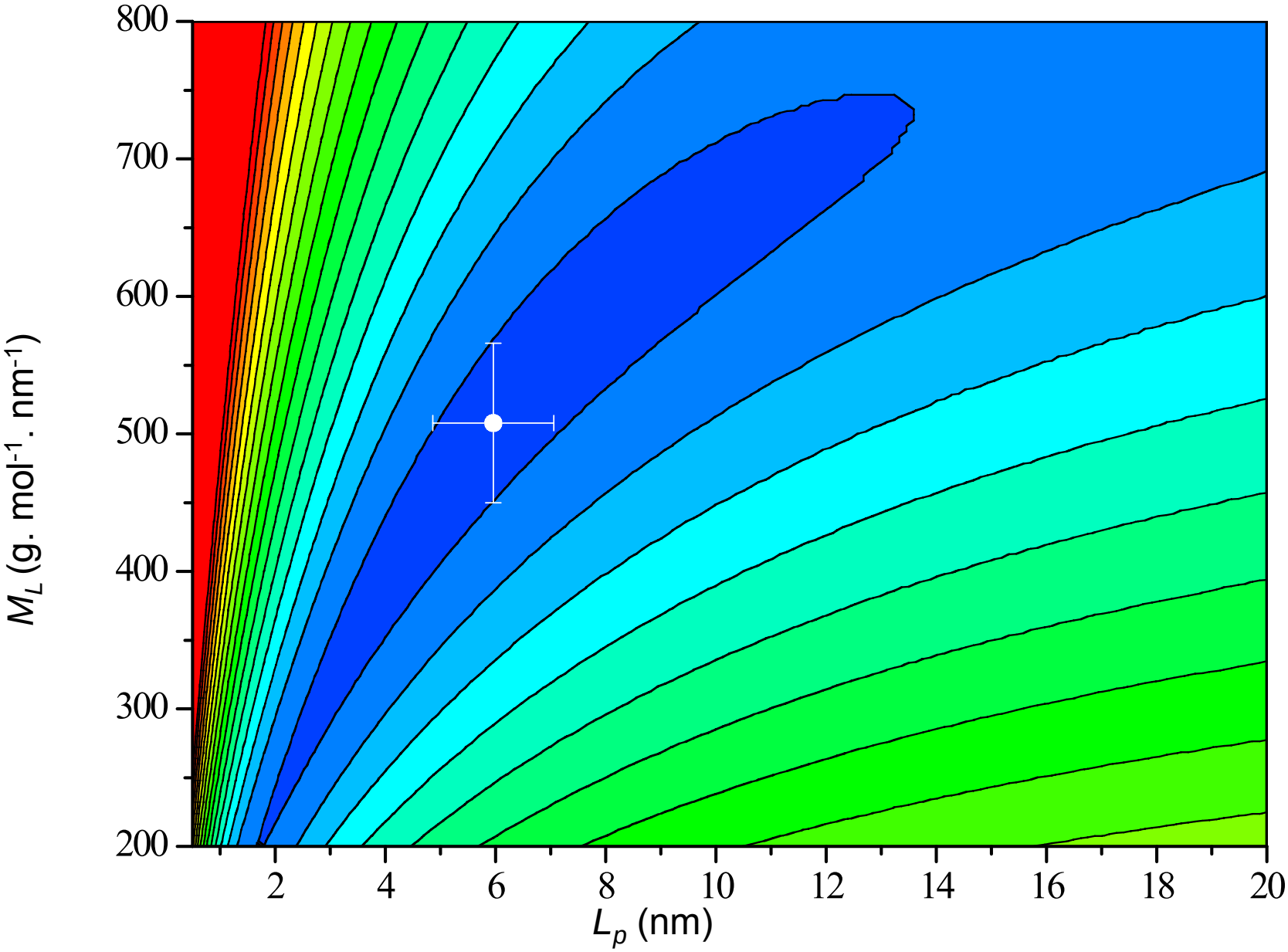
“Bushin-Bohdanecky” relation

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

“Yamakawa-Fujii” relation

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

Global “Hydfit” plot: xyloglucan

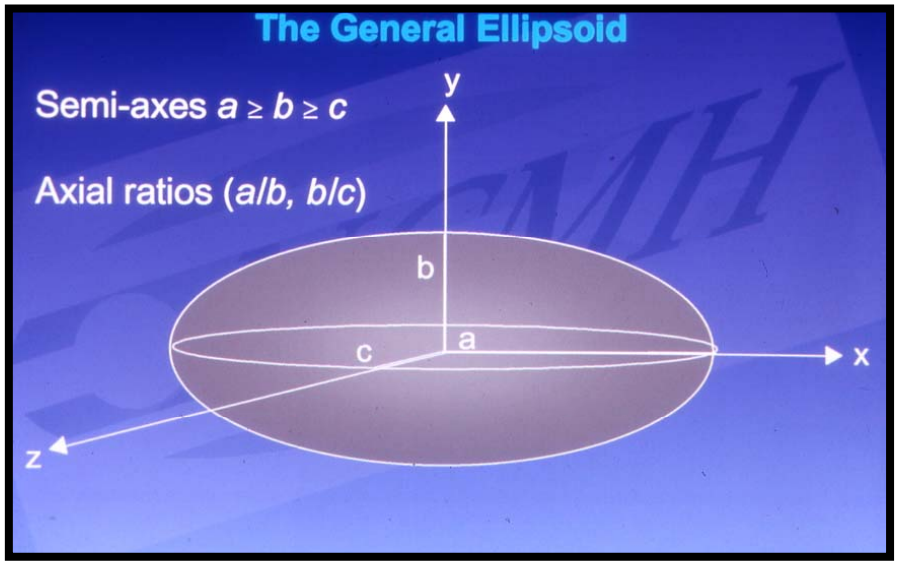


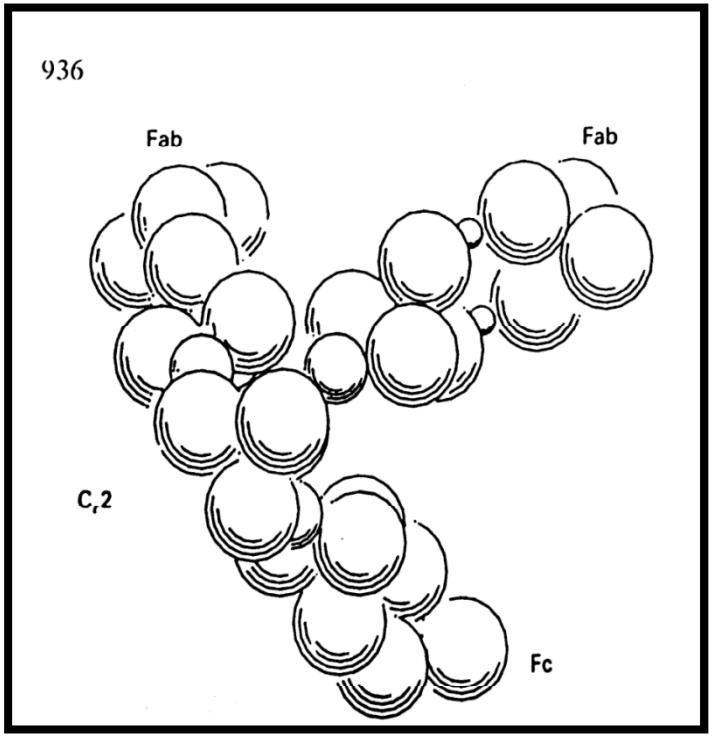
Patel et al, *Carbohydrate Polymers*, 2007

Flexibilities of carbohydrate polymers

Carbohydrate Polymer	L_p (nm)
Pullulan	1.2-1.9
Amylose	2.8
Pectin (69% esterified)	12-15
Pectin (0% esterified)	34
DNA	45
Schizophyllan	115-200
Scleroglucan	180 ± 30
Xanthan	210

Protein conformation: ellipsoids and beads

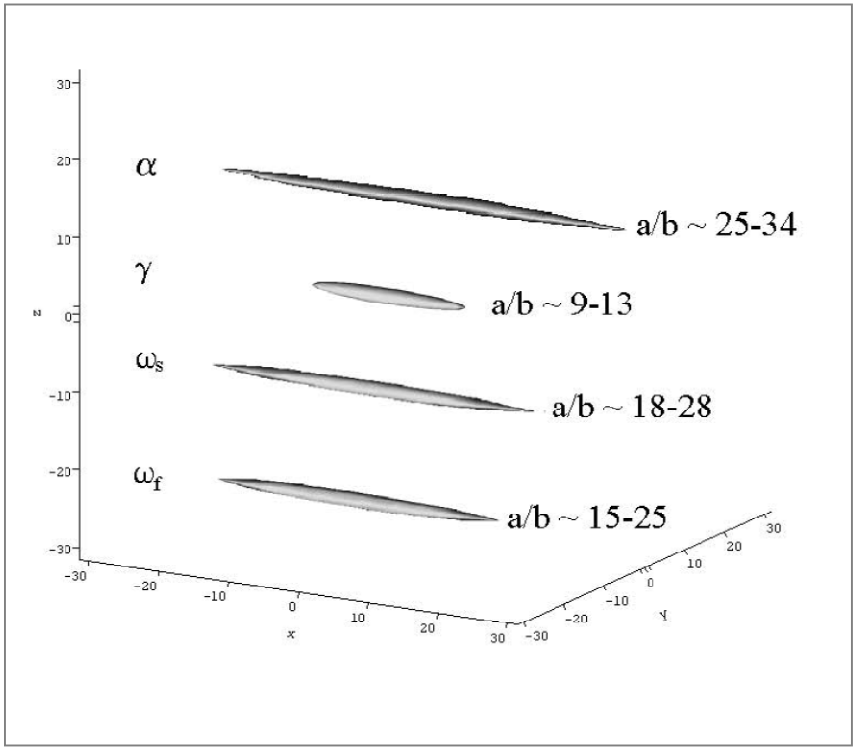




Software
http://www.nottingham.ac.uk/ncmh
Ellips1 (ellipsoids of revolution)
Ellips2, Ellips3, Ellips4 (general ellipsoids)

Software
http://leonardo.inf.um.es/macromol
Hydro,
Solpro,
HydroPro

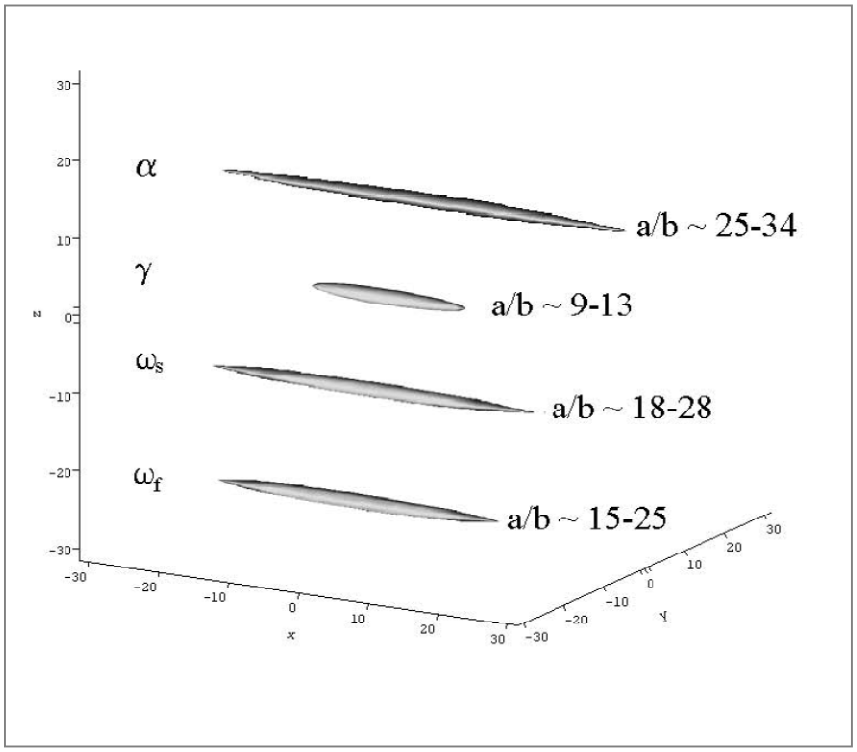
Ellipsoid axial ratio determinations – wheat protein gliadins



Structure and heterogeneity of gliadin: a hydrodynamic evaluation

S. Ang et al, Eur. Biophys. J. (2009)

Ellipsoid axial ratio determinations – wheat protein gliadins



Structure and heterogeneity of gliadin: a hydrodynamic evaluation
S. Ang et al, Eur. Biophys. J. (2009)

ELLIPS1

www.nottingham.ac.uk/ncmh

Demonstration of ELLIPS1 & ELLIPS2 programs:

download from http://www.nottingham.ac.uk/ncmh

For a wide variety of hydrodynamic parameters including ν (from intrinsic viscosity)– see Lecture 1 notes or P (from sedimentation or diffusion measurements) – see Lecture 3 notes:

$$\nu = [\eta] / v_s$$

$$P = (f/f_o).\ (v/v_s)^{1/3}$$

$$\text{where } (f/f_o) = (k_B T/6\pi\eta_o)\{(4\pi N_A/3\bar{v}M)^{1/3}\}/D^o_{20,w}$$

$$= (M(1-\bar{v}\rho_o)/N_A 6\pi\eta_o)\{(4\pi N_A/3\bar{v}M)^{1/3}\}/s^o_{20,w}$$

$$\frac{f}{f_0} = \frac{k_B T}{6\pi\eta_0}\left(\frac{4\pi N_A}{3\bar{v}M}\right)^{1/3} \cdot \frac{1}{D^\circ_{20,w}}$$

$$= \frac{M(1-\bar{v}\rho)}{N_A 6\pi\eta_0}\left(\frac{4\pi N_A}{3\bar{v}M}\right)^{1/3} \cdot \frac{1}{s^\circ_{20,w}}$$

$$\rho = \left(\frac{f}{f_0}\right) \cdot \left(\frac{\bar{v}}{v_s}\right)^{1/3}$$

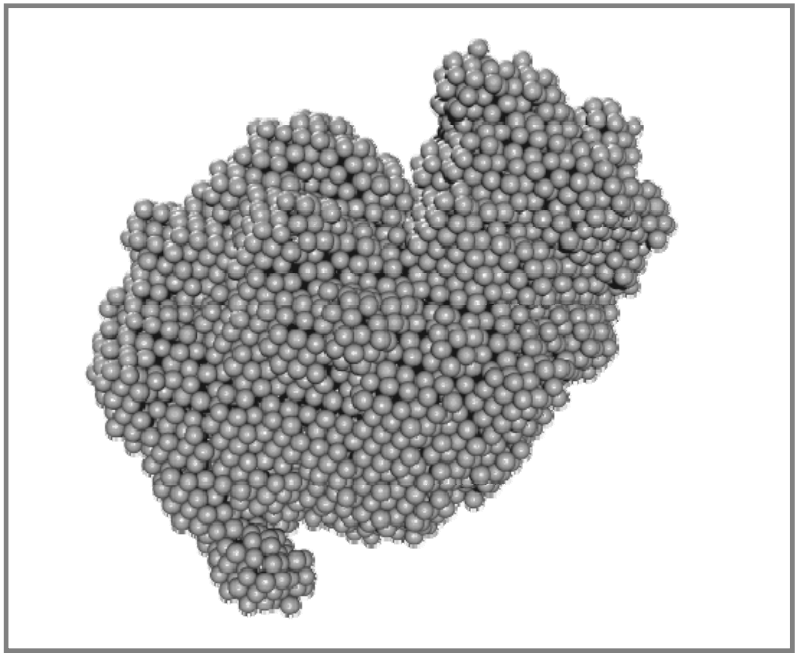
v_s : swollen specific volume (ml/g) $= \bar{v} + \delta/\rho_0$ (hydration)

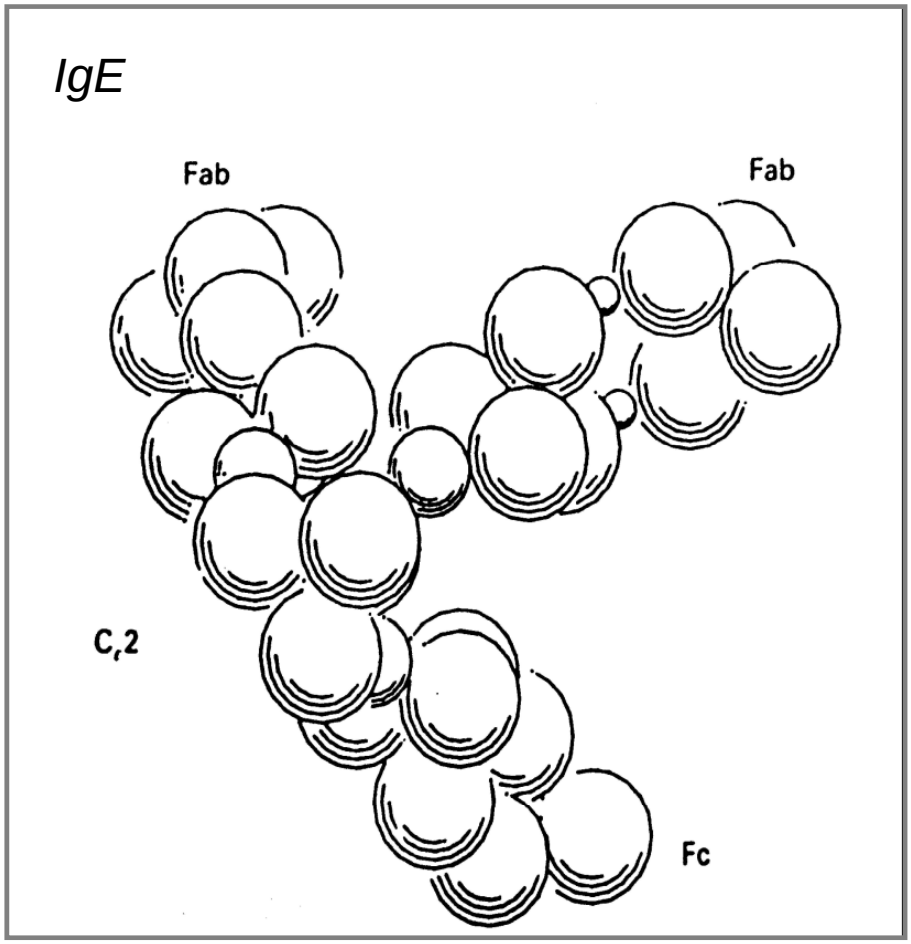
For more complicated shapes:

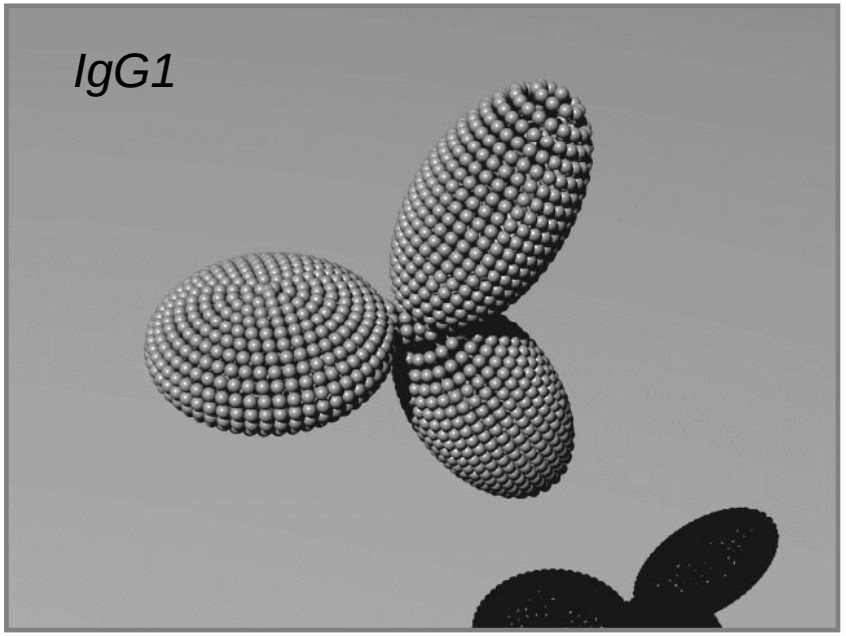
BEAD & SHELL MODELS

Hydro, Solpro, HydroPro etc

http://leonardo.inf.um.es/macromol/







Follow up bibliography:

1. Serydyuk, I.N., Zaccai, N.R. and Zaccai, J. (2006) *Methods in Molecular Biophysics*, Cambridge, Chapters D1 and D4
2. Harding, S.E., Rowe, A.J. and Horton, J.C., Eds (1992) *Analytical Ultracentrifugation in Biochemistry and Polymer Science,* Royal Soc. Chem. Cambridge
3. Scott, D.J. et al (2005) *Analytical Ultracentrifugation. Techniques and Methods*, Royal Soc. Chem. Cambridge
4. Harding, S.E. & Johnson, P.J. (1985) The concentration dependence of macromolecular parameters, *Biochem. J.* 231, 543-547
5. Harding, S.E. (1995) On the hydrodynamic analysis of macromolecular conformation. *Biophys Chem* 55, 69-93
6. Garcia de la Torre et al (1997) SOLPRO: theory and computer program for the prediction of SOLution PROperties of rigid macromolecules and bioparticles. *Eur. Biophys. J.* 25, 361-372