Hydrodynamic Characterisation

Viscometry

SEC-MALLs

Analytical Ultracentrifugation

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NCMH at Nottingham: A Facility for characterising sizes/shapes and interactions of macromolecules in solution

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

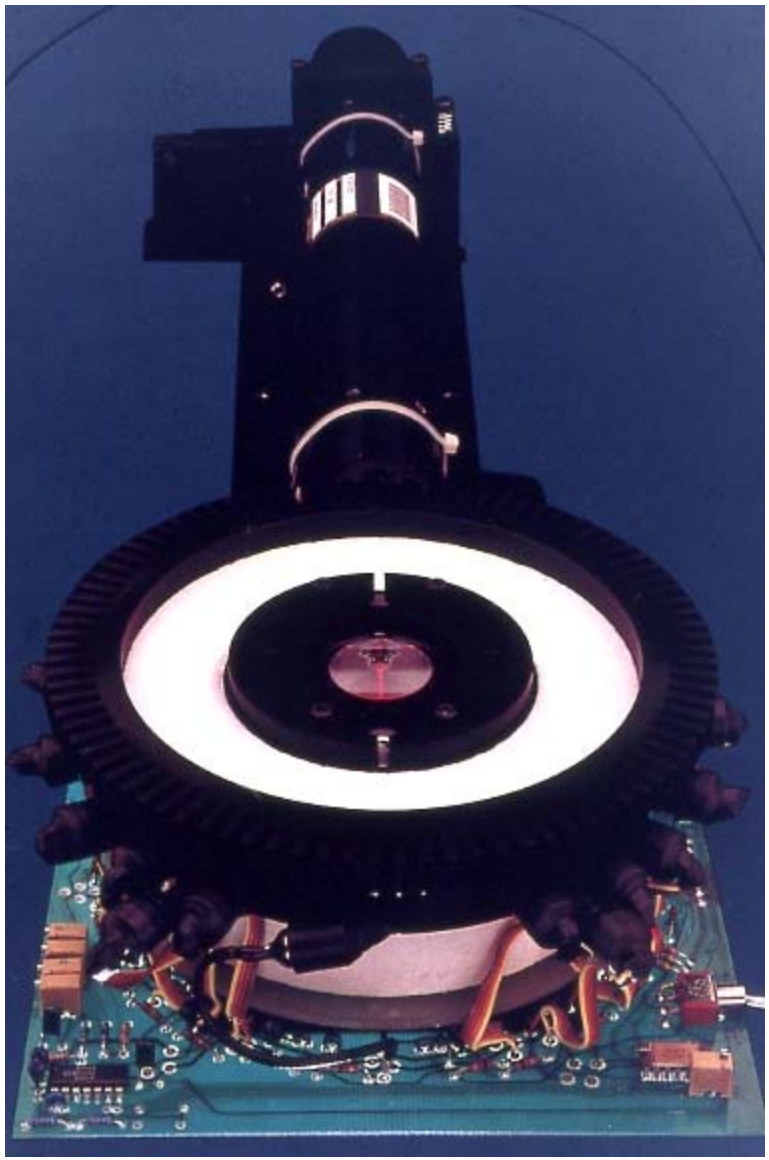
1. *Molecular weight distribution analysis – SEC MALLs and AUC*

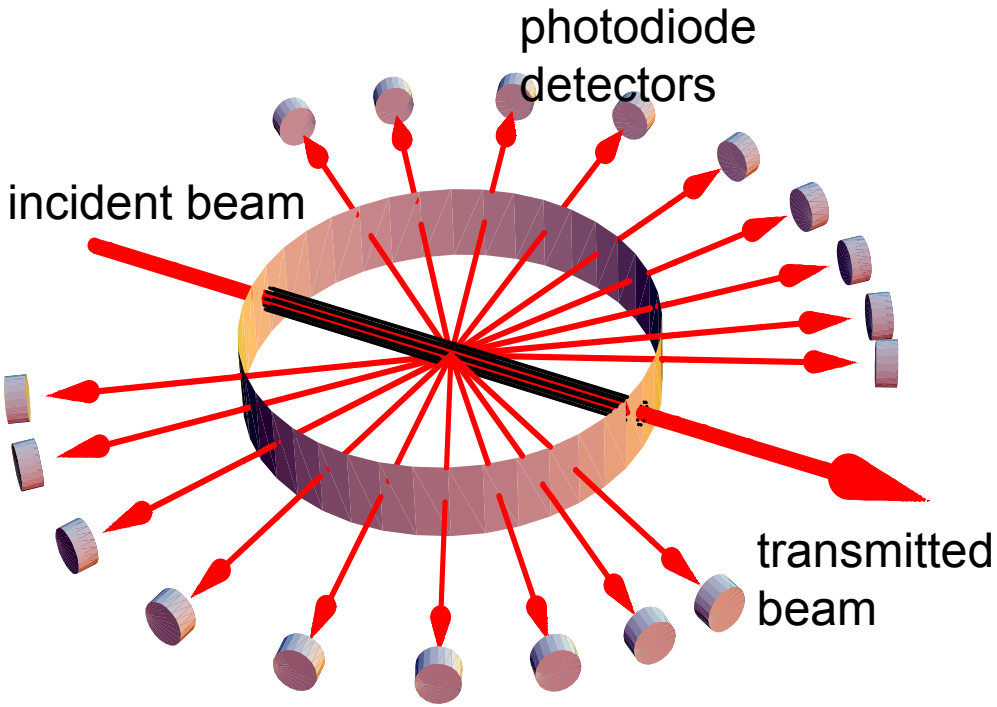
2. *Conformation and flexibility – Viscometry, AUC, Light scattering*

Molecular Weight: Light scattering

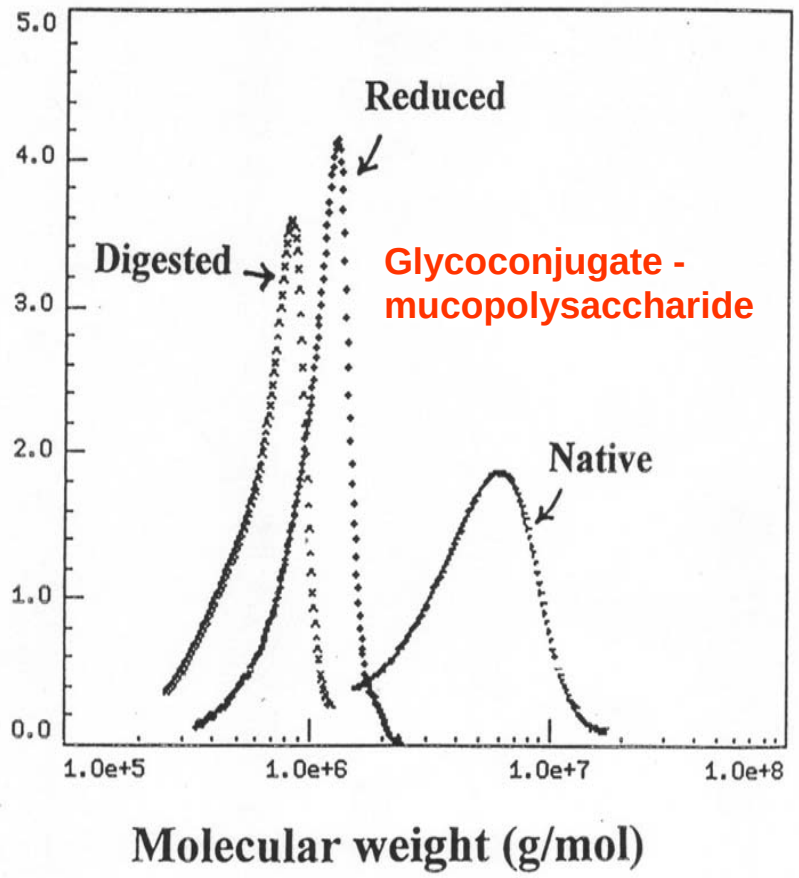
“MALLs” detector

Molecular Weight: Light scattering





Molecular Weight: SEC–MALLS



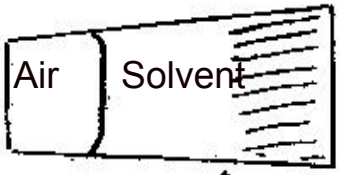
Fogg FJJ et al, *Biochemical Journal.* 1996

Molecular weight: analytical ultracentrifugation

Analytical ultracentrifugaton:

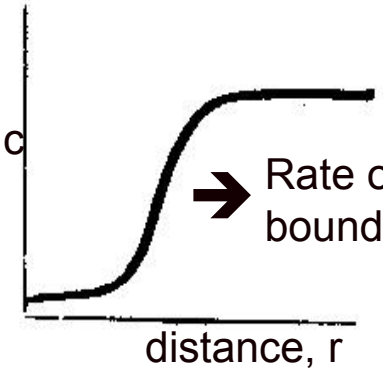
Sedimentation Velocity

Centrifugal force ➔



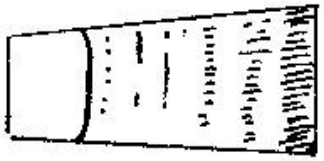
Top view, sector of centrifuge cell

Solution

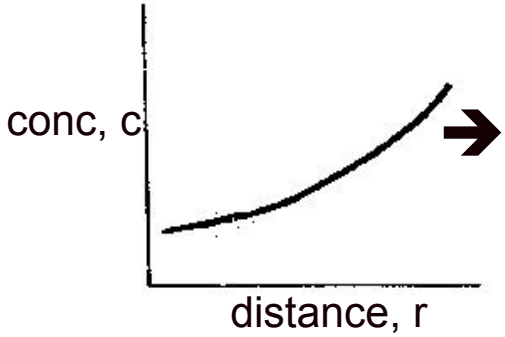


➔ Rate of movement of boundary → sed. coeff

Sedimentation Equilibrium

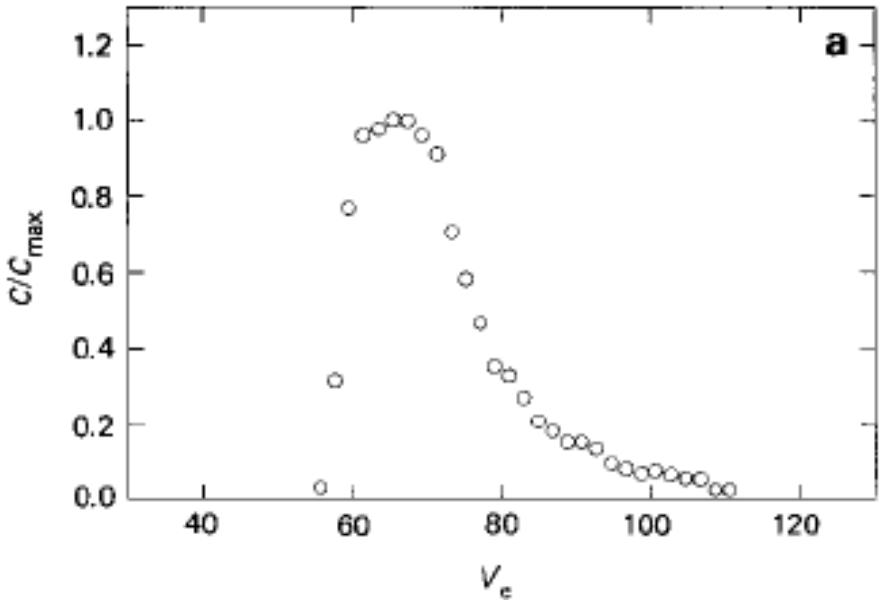


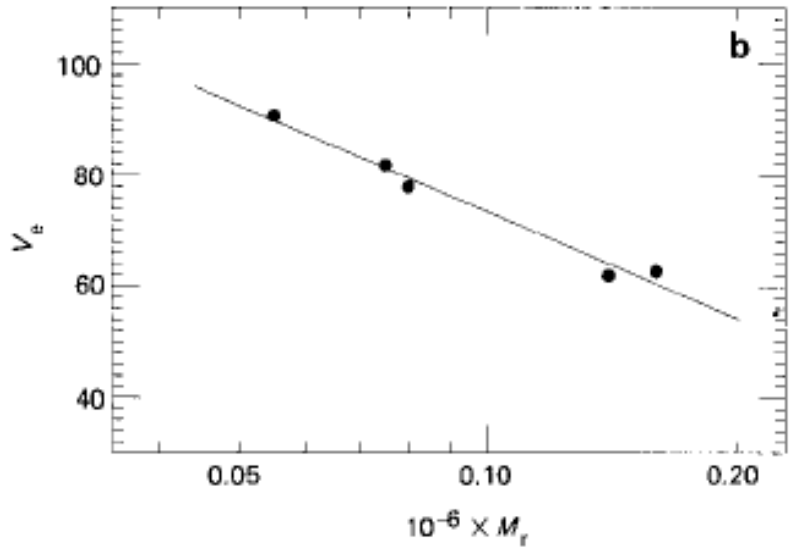
Centrifugal force ➔
➔Diffusion

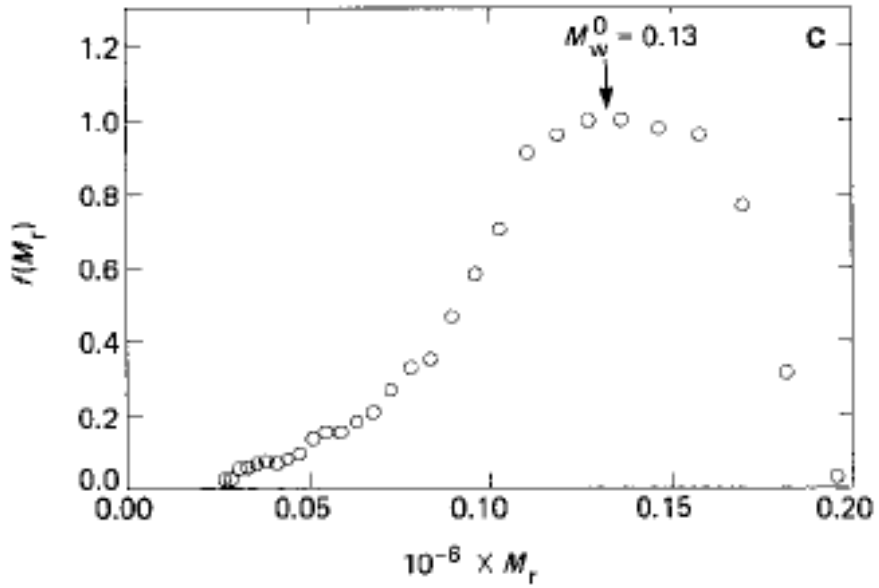


STEADY STATE PATTERN

FUNCTION ONLY OF MOL. WEIGHT PARAMETERS







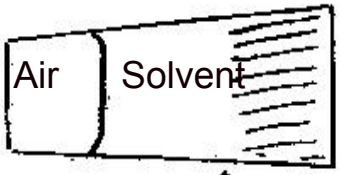
SEC - sedimentation equilibrium

mol. wt distribution: alginate

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

Sedimentation Velocity

Centrifugal force ➔



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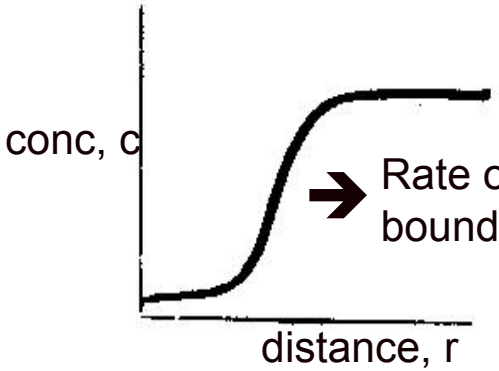
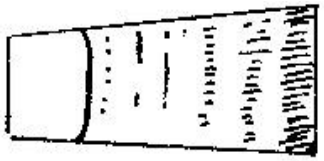
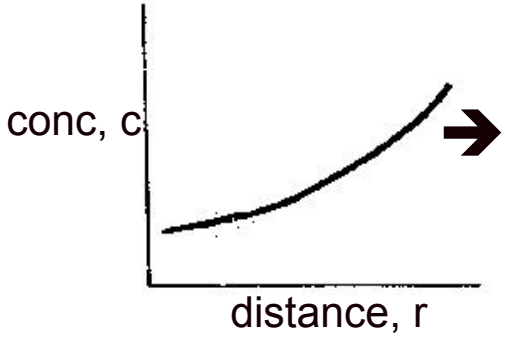


➔ Rate of movement of boundary → sed. coeff

Sedimentation Equilibrium



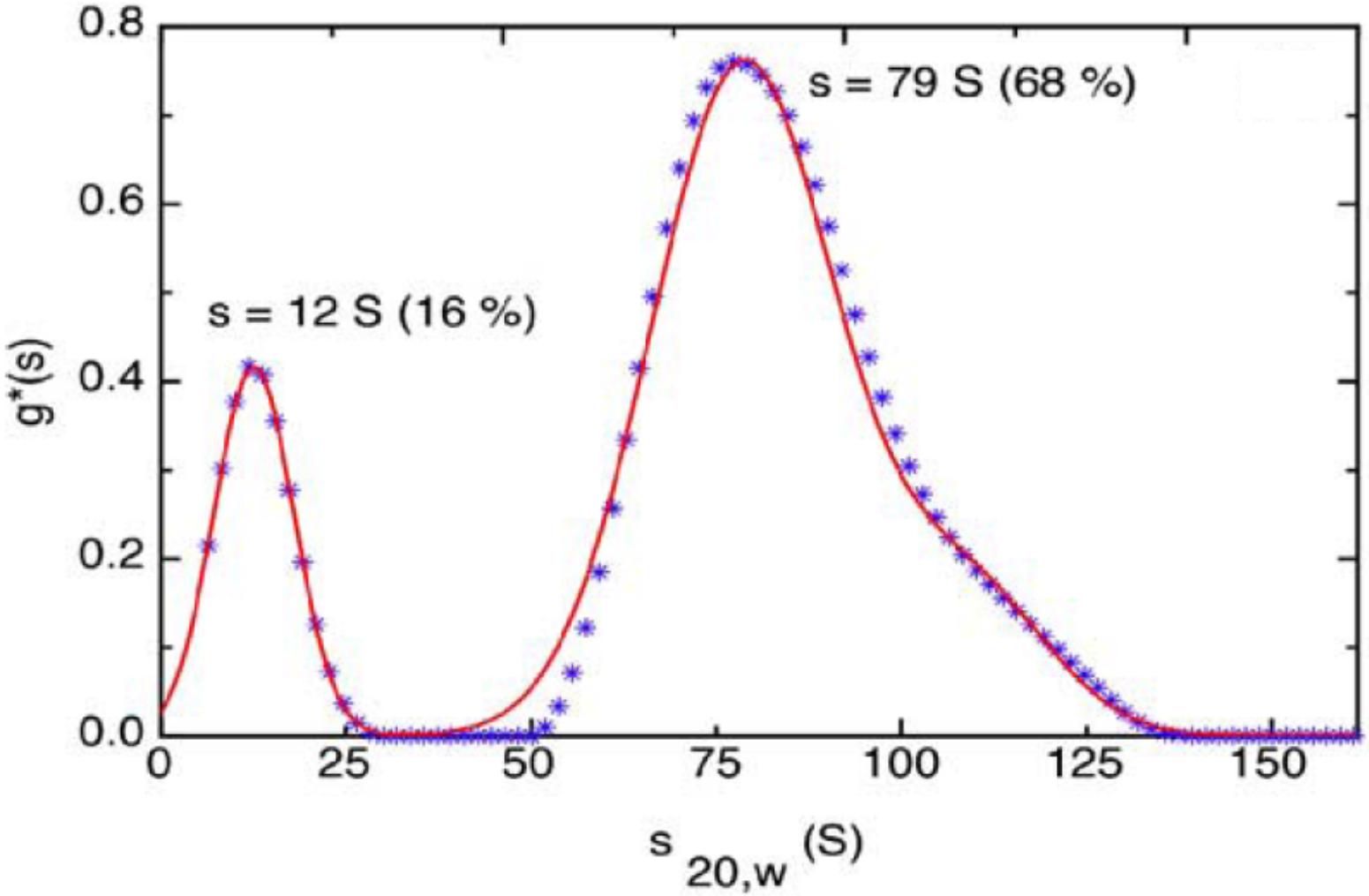
Centrifugal force ➔
➔Diffusion



STEADY STATE PATTERN

FUNCTION ONLY OF MOL. WEIGHT PARAMETERS

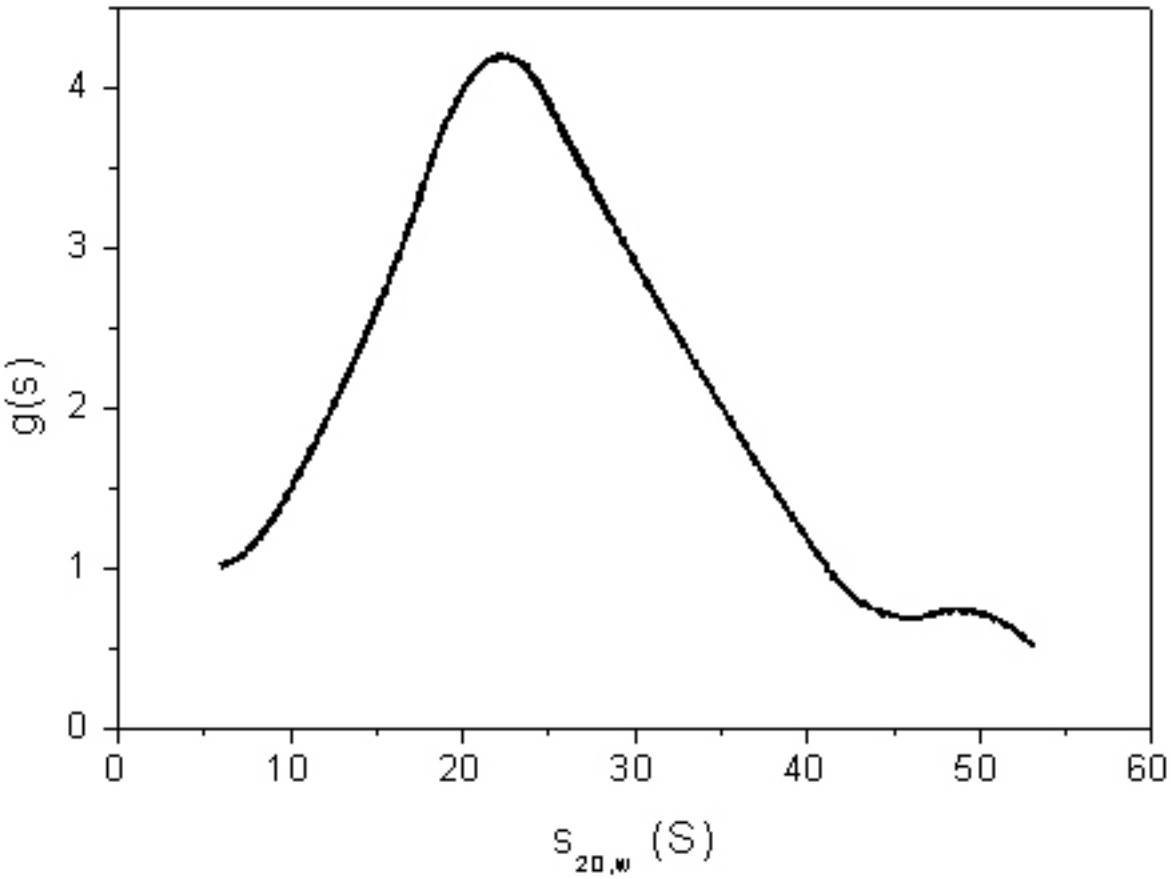
Sedimentation velocity g(s) plot: starch*



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

Converting a sedimentation coefficient distribution to a molecular weight distribution

Gycoconjugate: Mucopolysaccharide

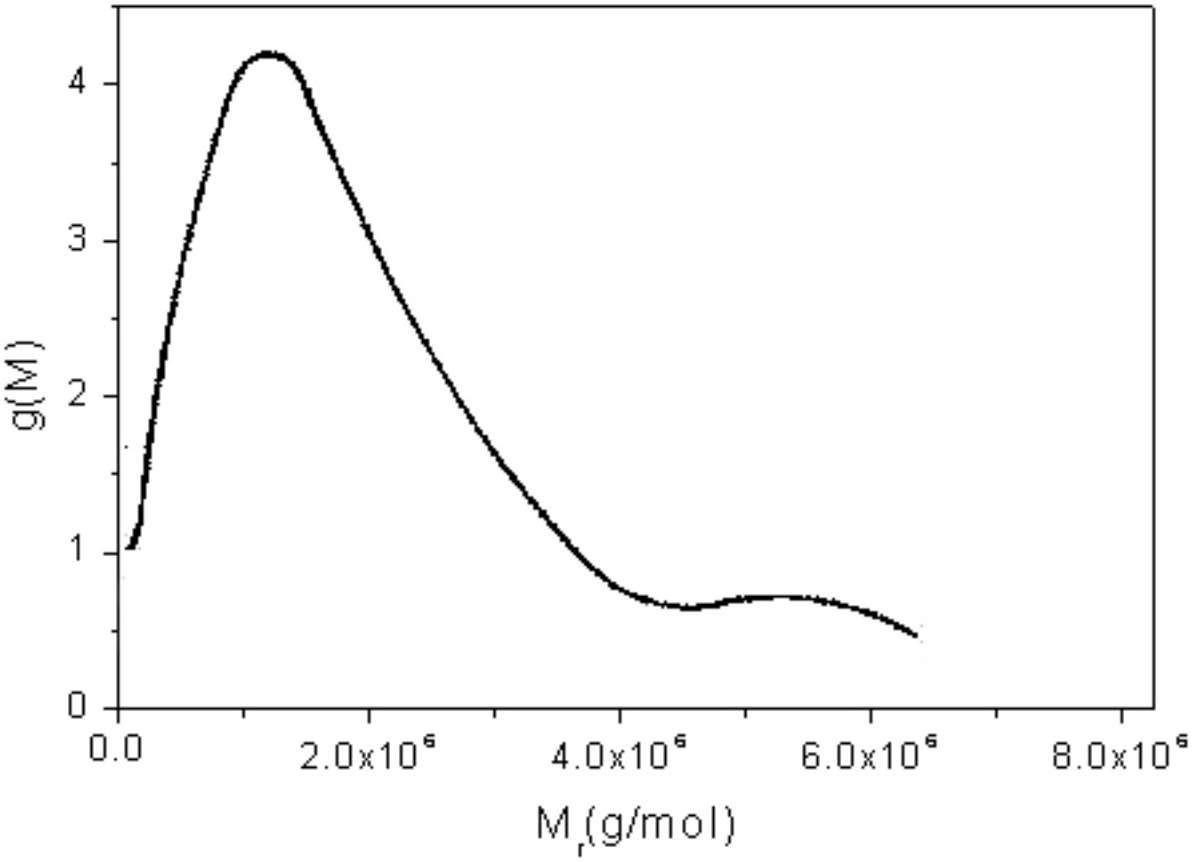


$s_{20,w} \sim M^b$

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

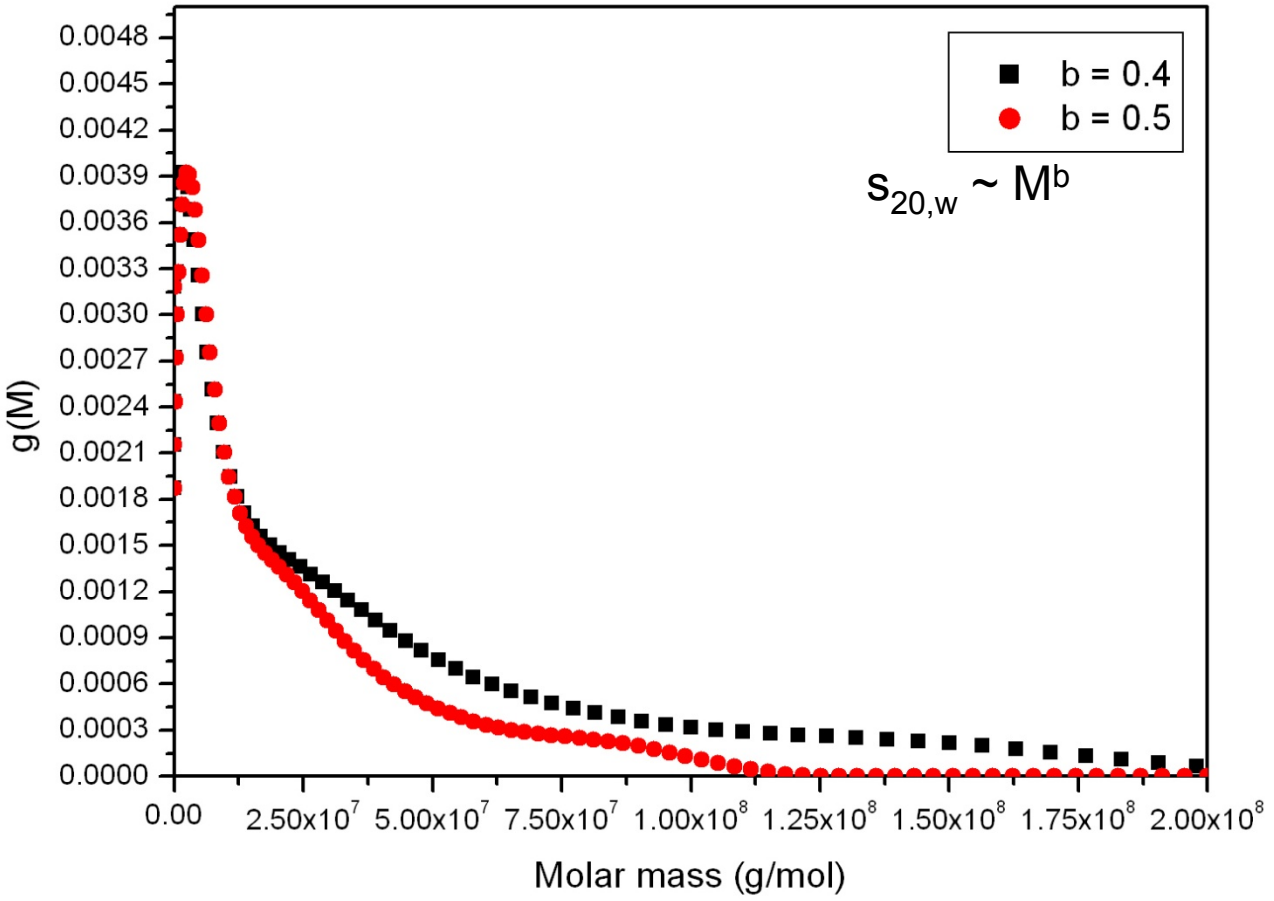


$s_{20,w} \sim M^b$ (b=0.5)

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

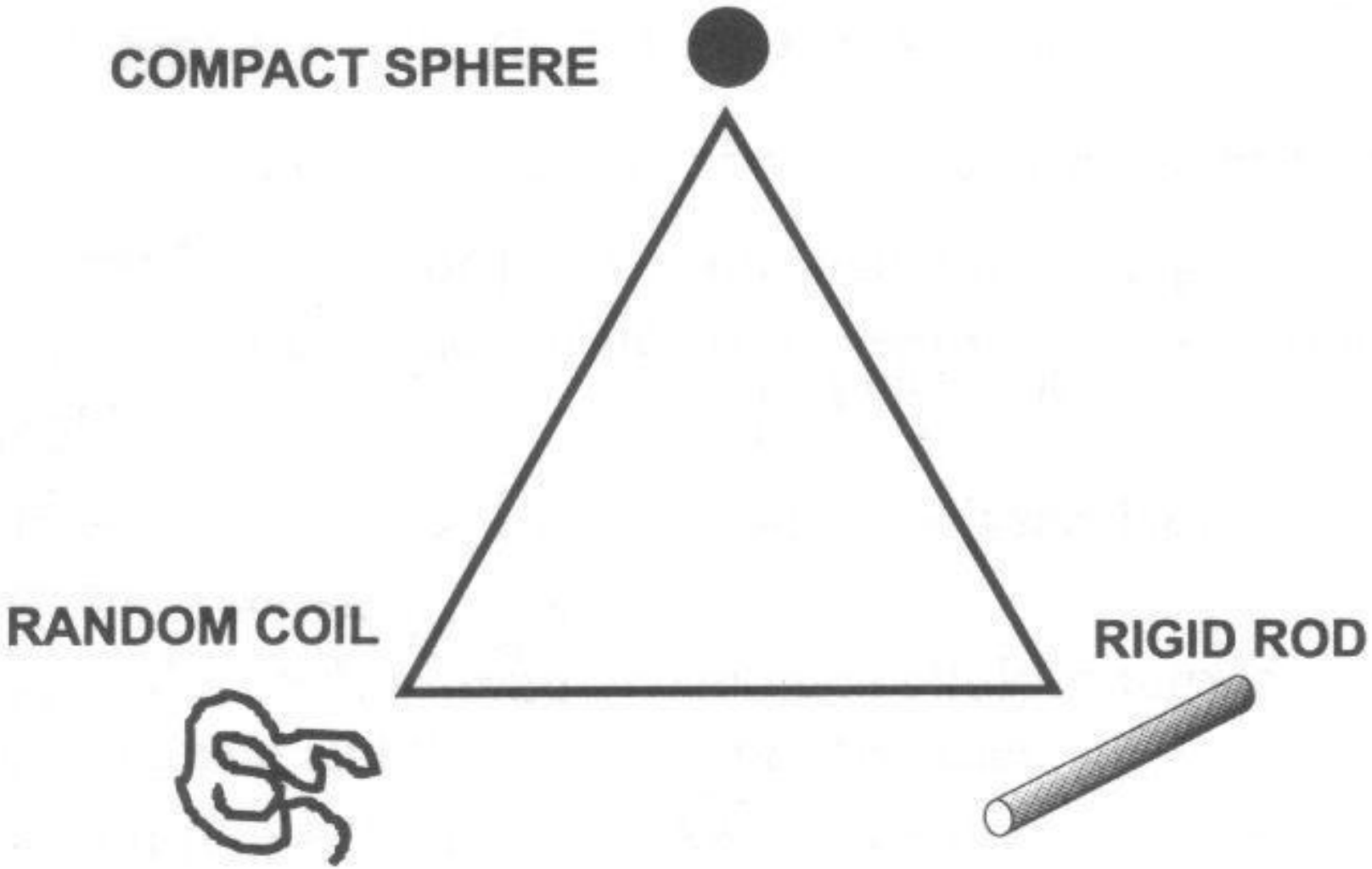


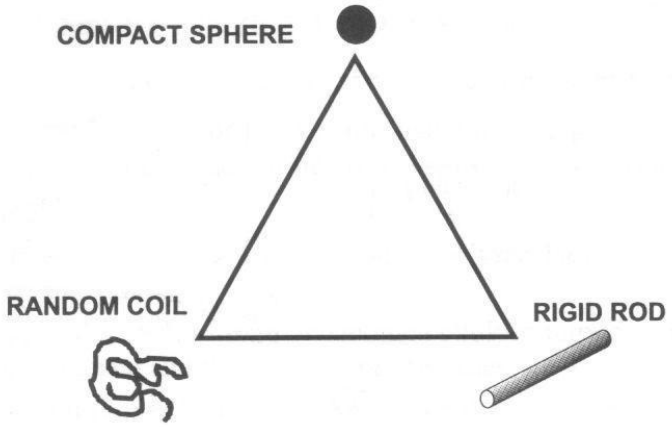
Harding, S., Abdelhameed, A., Morris, G. (2010)

1. *Molecular weight distribution analysis – SEC MALLs and AUC*

2. *Conformation and flexibility – Viscometry, AUC, Light scattering*

Haug Triangle





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

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

Conformation Zoning:

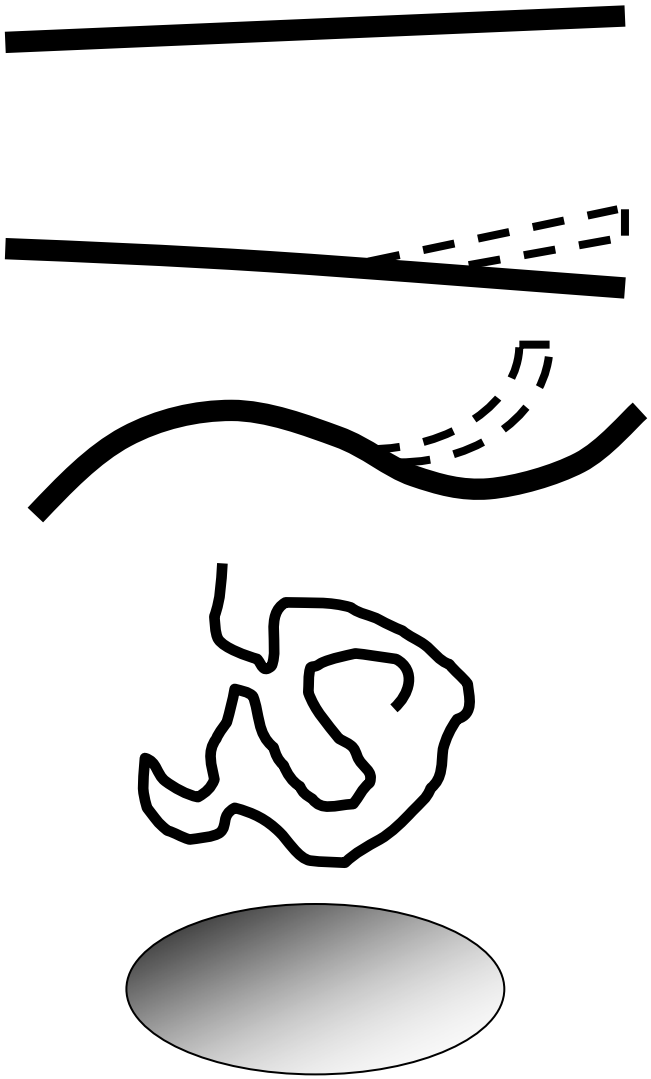
Zone A: Extra-rigid rod: schizophyllan

Zone B: Rigid Rod: xanthan

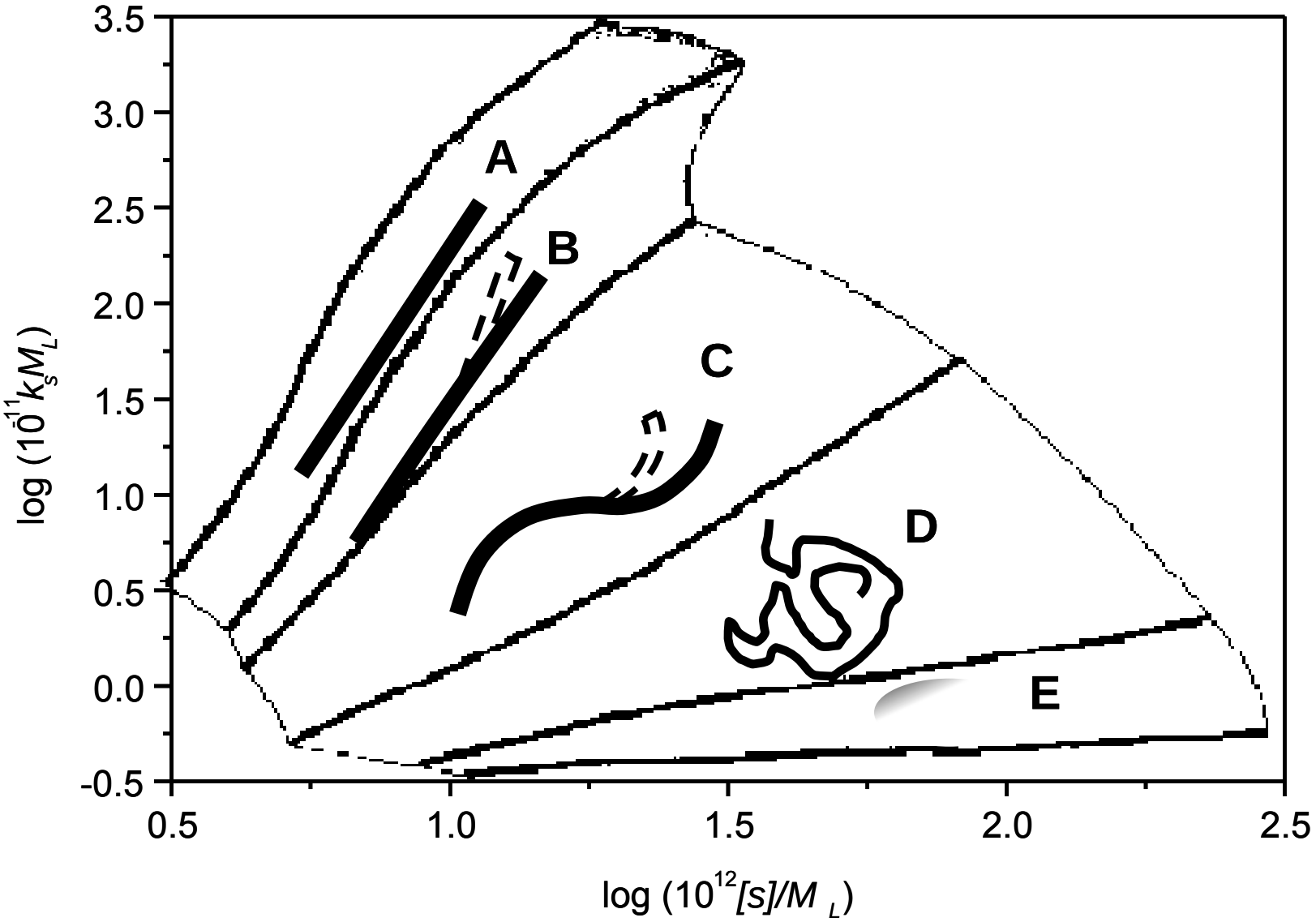
Zone C: Semi-flexible coil: pectin

Zone D: Random coil: dextran, pullulan

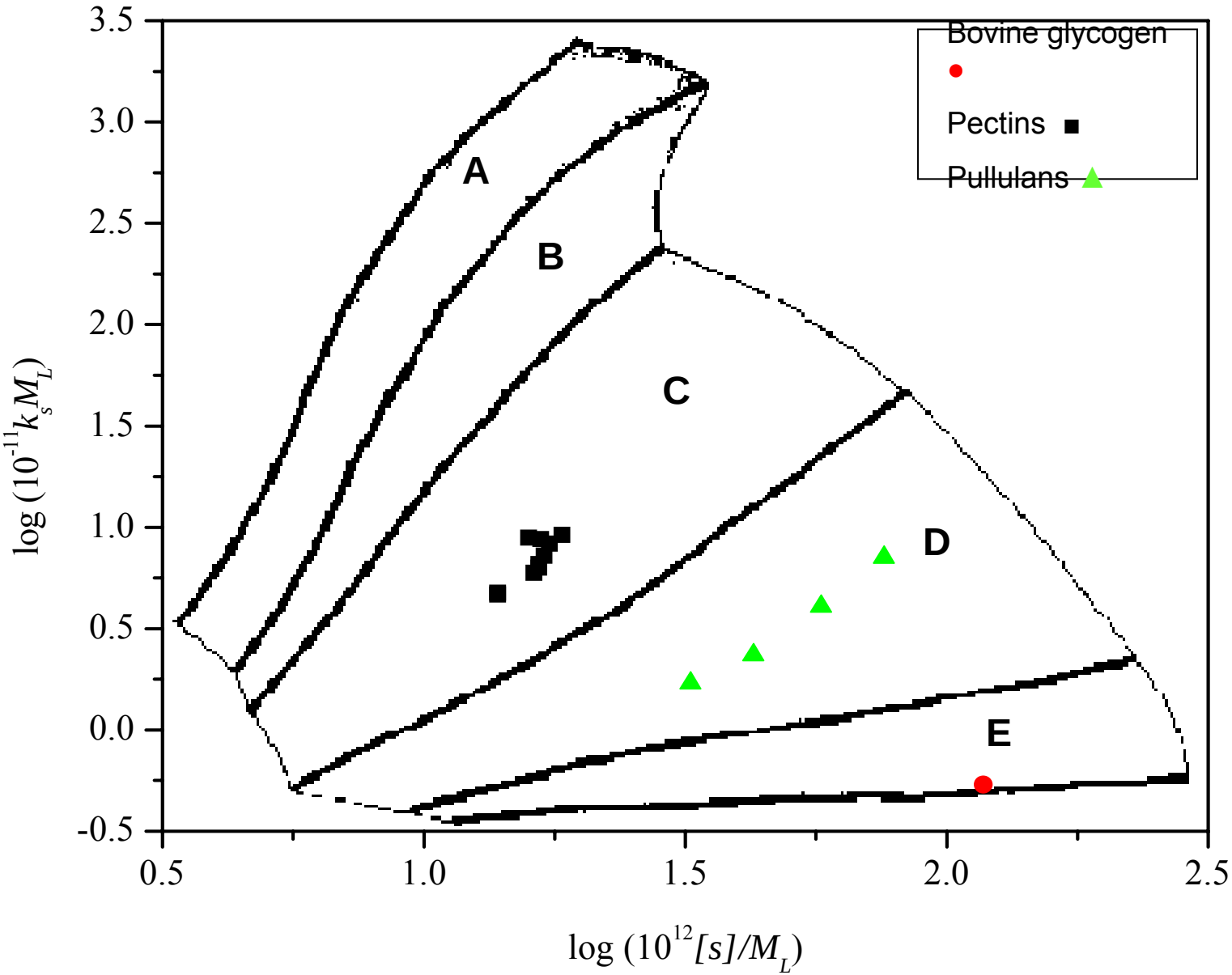
Zone E: Highly branched: amylopectin, glycogen



Conformation Zoning:

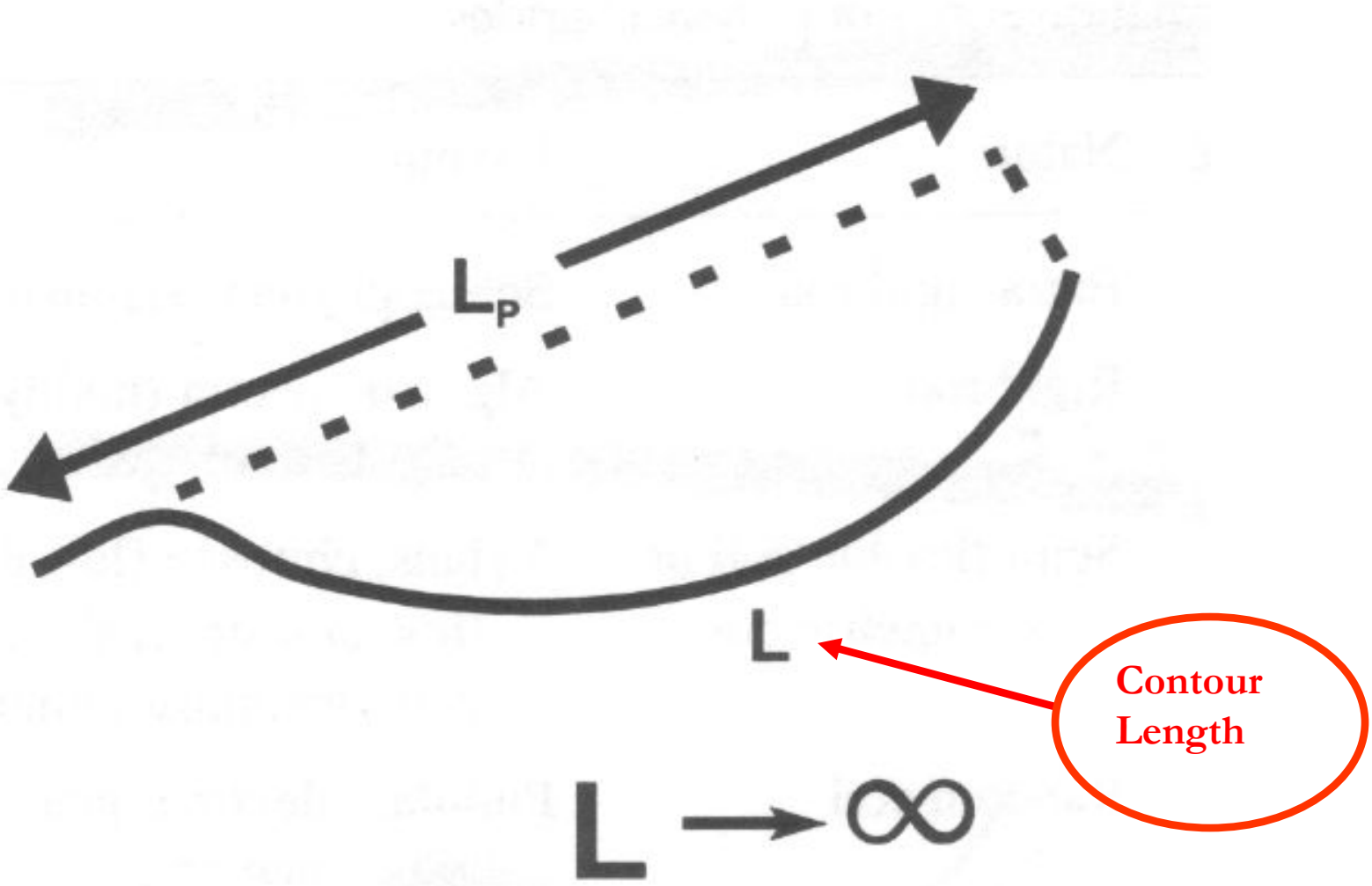


Pavlov, Rowe and Harding. *Trends in Analytical Chemistry*,1997


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

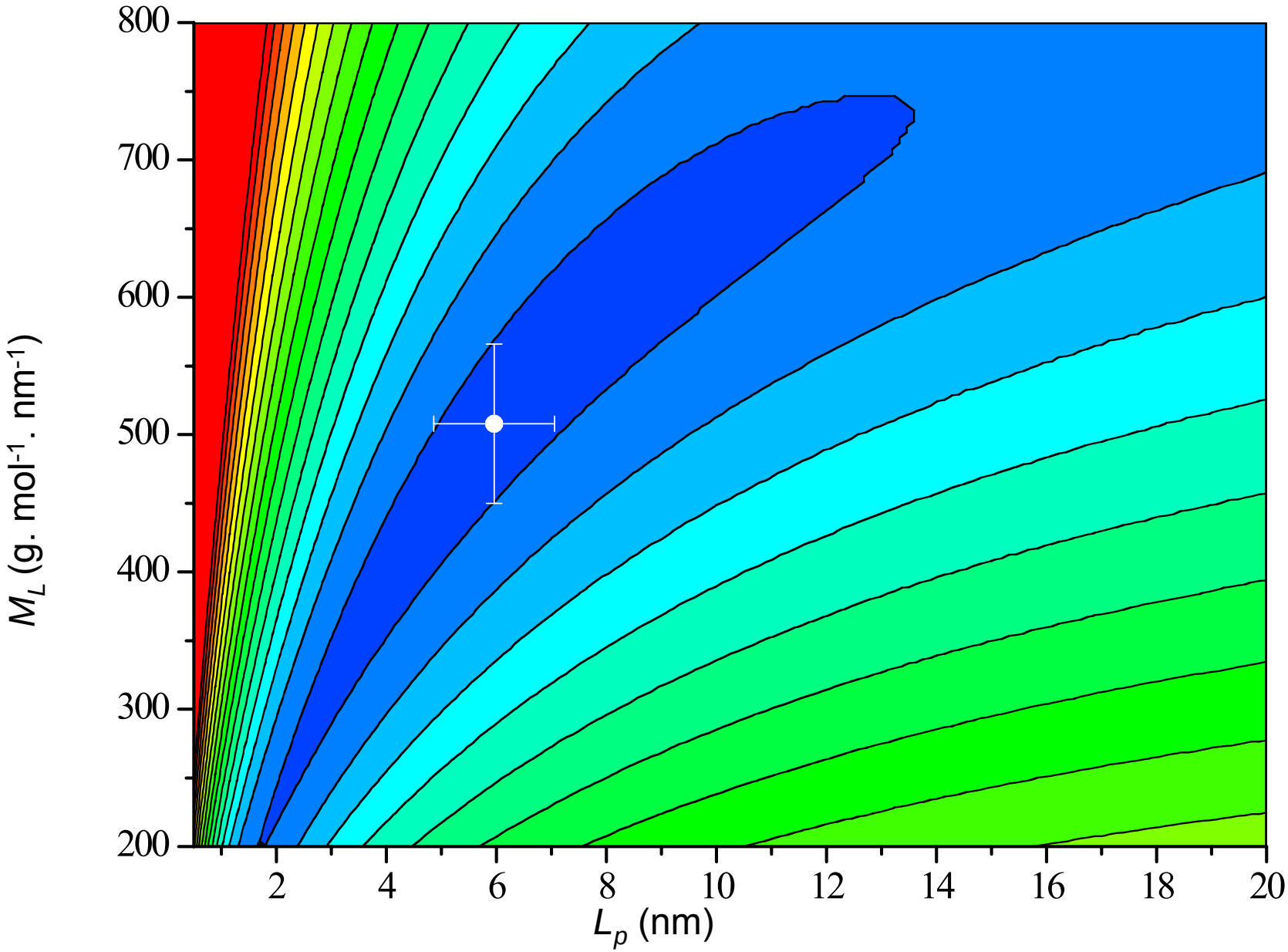
“Bushin-Bohdanecky” relation

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

“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

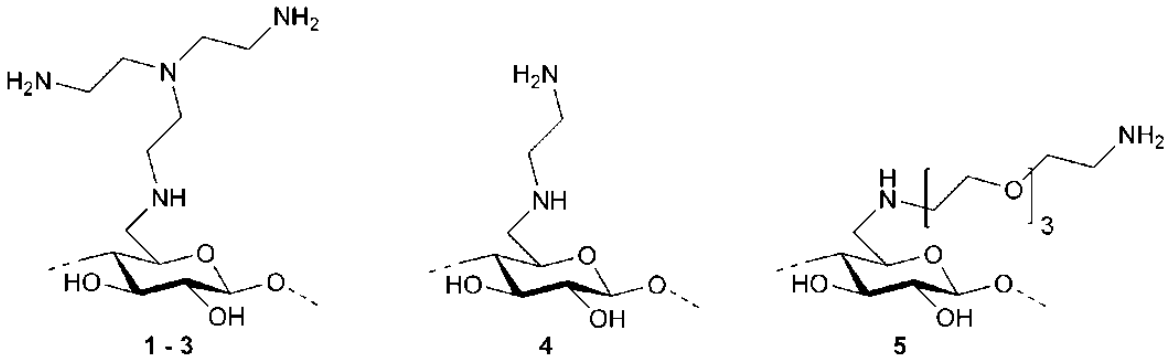


Patel et al, *Carbohydrate Polymers*, 2007

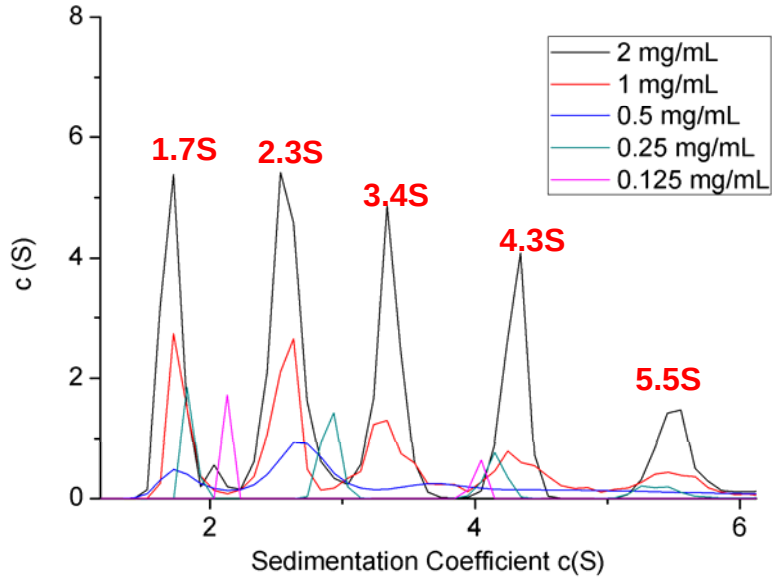
Flexibilities of carbohydrate polymers

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-like self-association in amino-celluloses!



all M_1 ~ 20kDa



$s \sim M^{2/3}$

Hydrodynamic characterisation - summary

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2. *Conformation and flexibility – Viscometry, AUC, Light scattering*