Study by Analytical Centrifugation on Konjac glucomannan

Ali Saber Abdelhameed and Stephen Harding

NCMH, University of Nottingham

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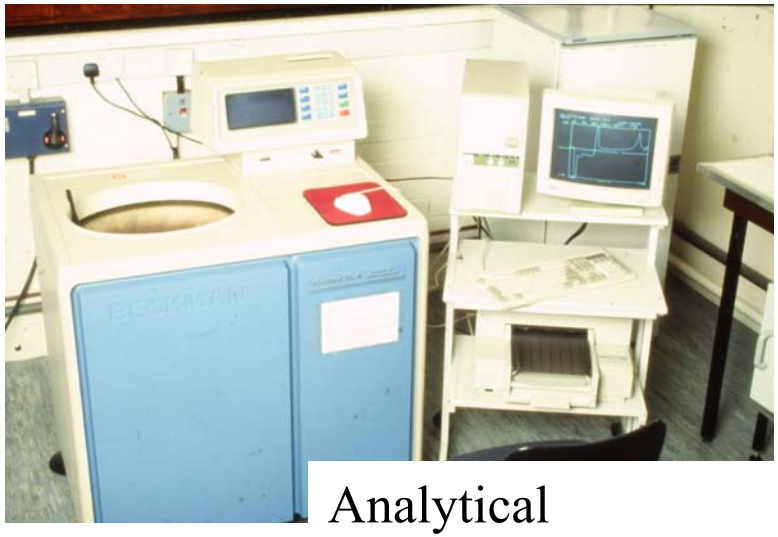

Analytical Ultracentrifugation

SEC-MALLs

Viscometry

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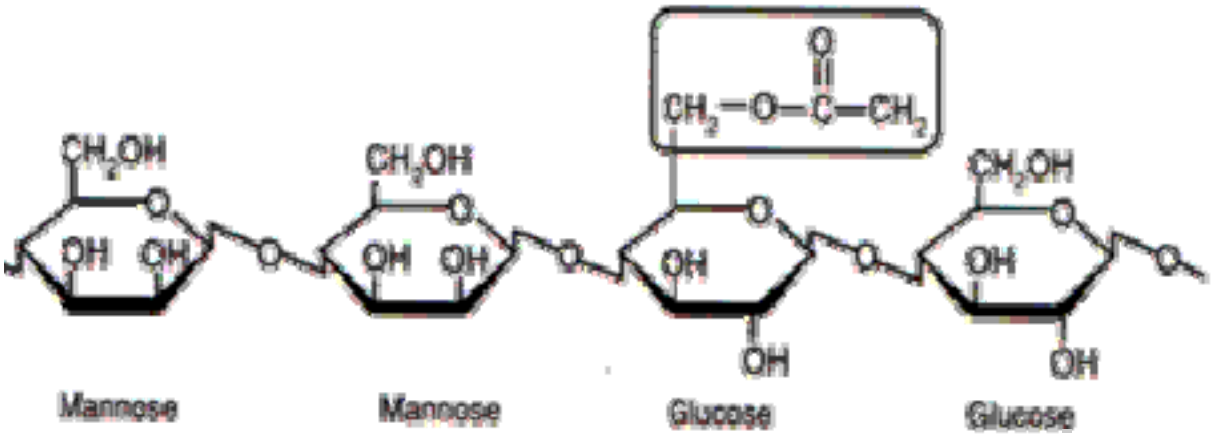
Konjac Glucomannan



http://img.nextag.com/image/JARROW-Formulas-Glucomannan-120/1/000/005/511/883/551188378.jpg

ELSEVIER

S0144-8617(96)00004-5

Carbohydrate Polymers **28** (1995) 325–332

0144-8617/95/$09.50+.00

Characterization of gliadin–galactomannan incubation mixtures by analytical ultracentrifugation— Part I. Sedimentation velocity

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The aim of this work is to examine the possible interaction and extent thereof of the polysaccharide galactomannan (GAL) with the cereal protein gliadin (GLI) and a peptic–tryptic degraded gliadin (PT–GLI) by analytical ultracentrifugation. The work is part of a series of investigations into the field of coeliac disease (gluten-induced enteropathy) as gliadins are known to be toxic for patients with this disease.

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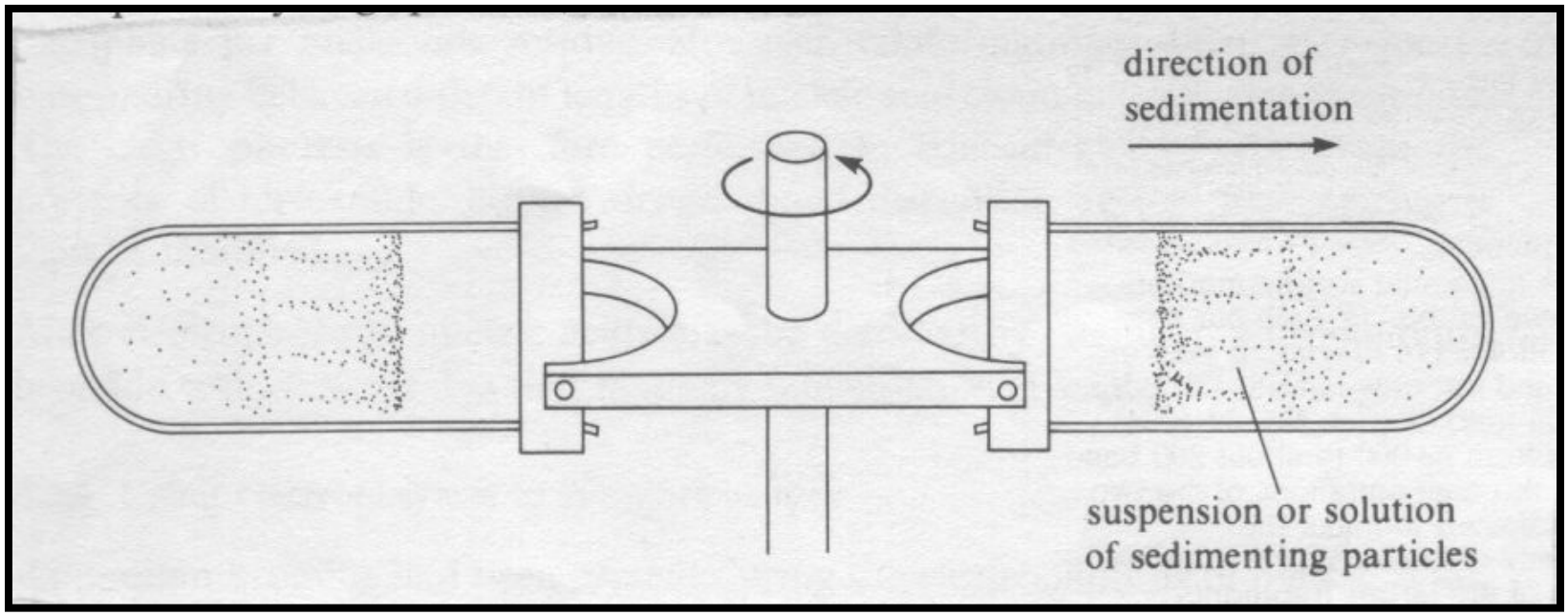
The molecular integrity of the GAL and GLI preparations was first checked by sedimentation velocity and sedimentation equilibrium. Sedimentation velocity showed single boundaries indicating homogeneity and low-speed sedimentation equilibrium gave plausible apparent weight average molar masses of 180,000 g/mol for GAL and 20,000 g/mol for GLI. PT–GLI, GLI and GAL in phosphate buffer (pH 6.5) and the incubated mixtures (stirred for 3 h at 37°C; PT–GLI:GAL = 3.53:1, wt.wt.; GLI:GAL = 0.23 and 0.55:1, wt.wt.) were then investigated by sedimentation velocity at a temperature of 20°C. The plots of $1/s_{20}$ vs. c of GAL, PT–GLI–GAL and GLI–GAL mixtures after incubation show a significantly different shape suggesting the presence of interactions. According to the equation $1/s_{20} = 1/s^{\circ}_{20}(1 + k_s c)$, values for $\{s^{\circ}_{20}, k_s\}$ of {(4.02±0.23) S, (490.9±28.9) ml/g}, {(5.92±0.24) S, (1152±44) ml/g} and {(5.38±0.19) S, (1141±38) ml/g} for GAL and PT–GLI–GAL and GLI–GAL mixtures, respectively, were obtained. The concentration of GAL ranged from 0.75–3.0 mg/ml for GAL alone and from 0.34–1.50 mg/ml in the incubated mixtures. This apparent indication for a weak non-covalent protein–polysaccharide interaction was further supported by UV absorption spectrometry and gel filtration.

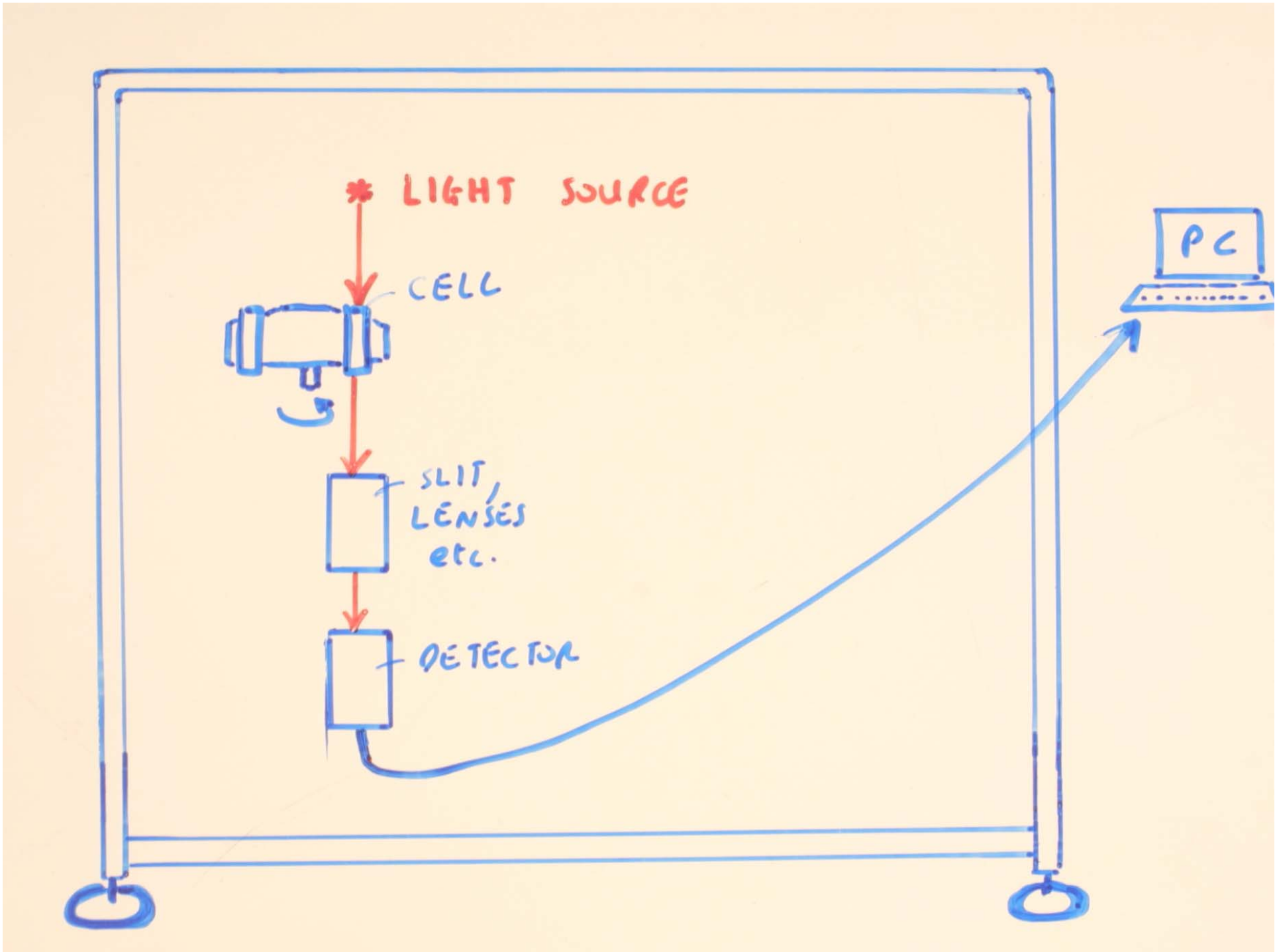
Physical characterisation

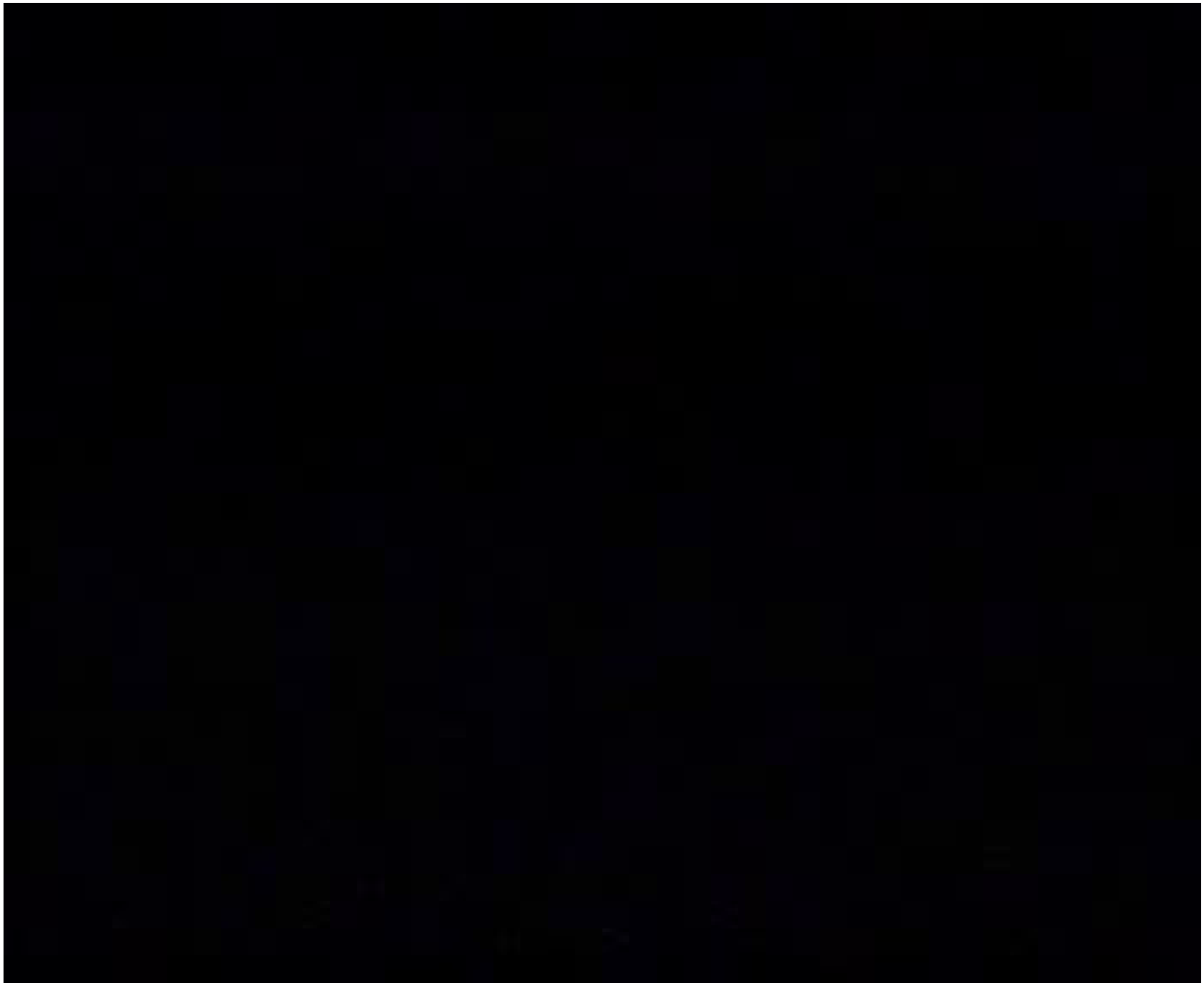
1. Heterogeneity: Sedimentation coefficient and distribution. Molecular weight & distribution.
2. Intrinsic Viscosity.. and distribution
3. Conformation in solution, flexibility
4. [Interactions]

Analytical Ultracentrifugation

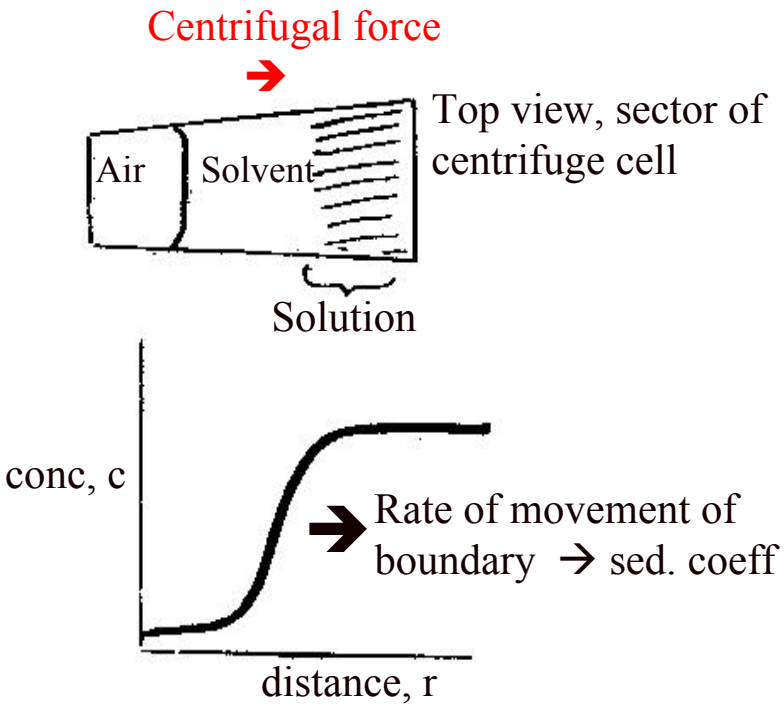


Optima XLA/ XLI


LIGHT SOURCE
CELL
SLIT, LENSES etc.
DETECTOR
PC



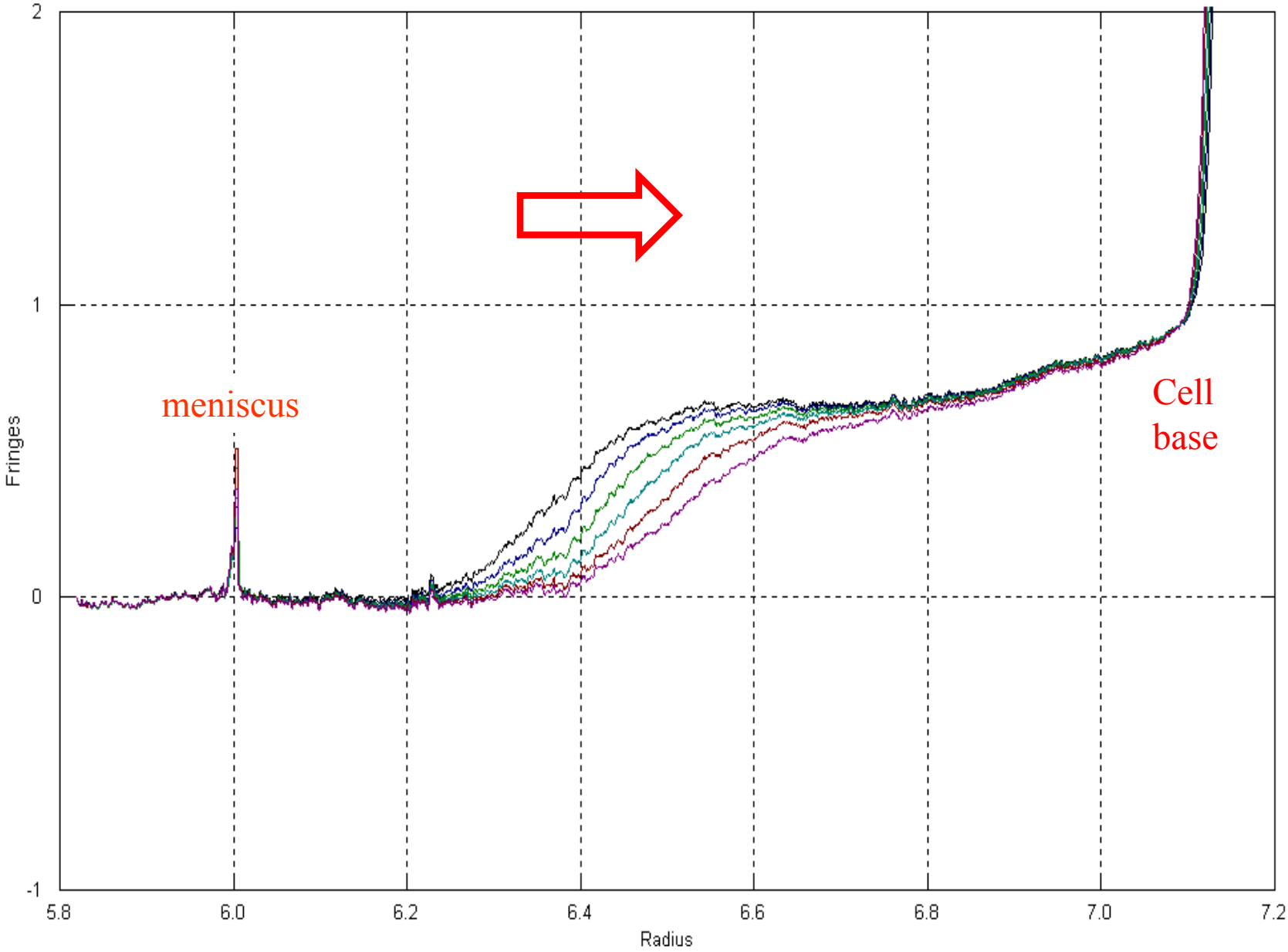
Sedimentation Velocity



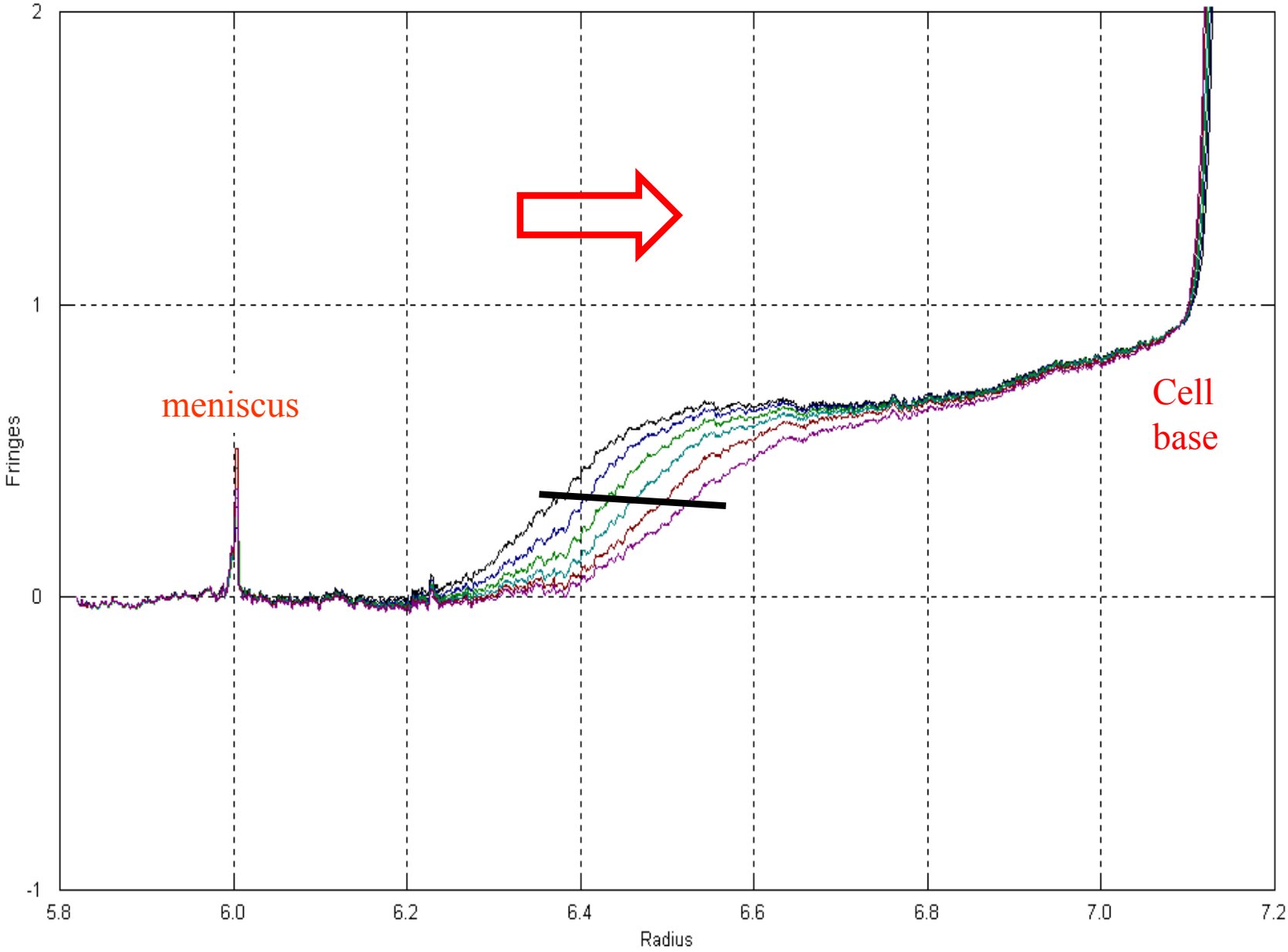
$$\Rightarrow \quad s^{o}_{20,w}$$

Sedimentation coefficient, S

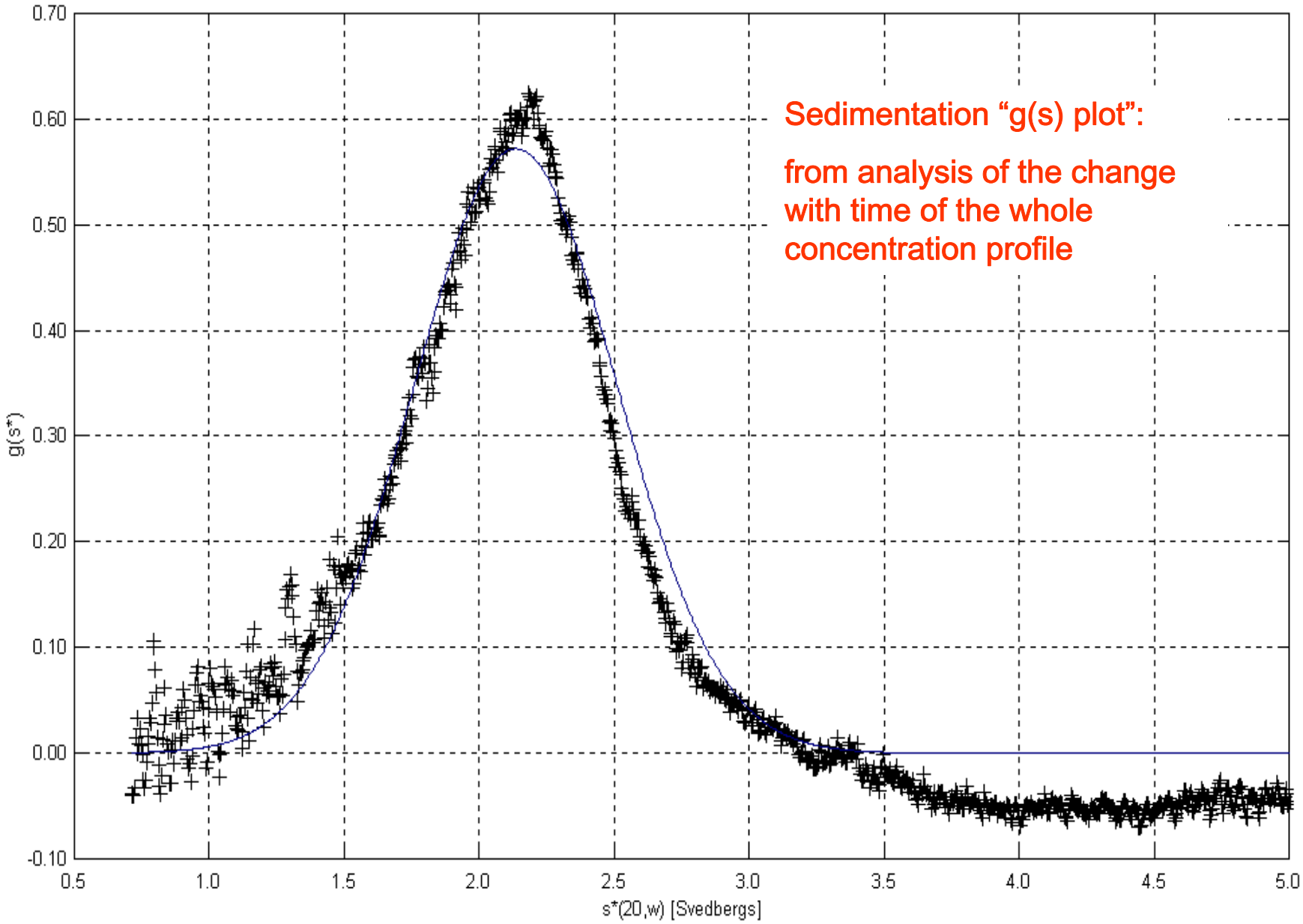
Chitosan G213, 0.5 mg/ml



Chitosan G213, 0.5 mg/ml



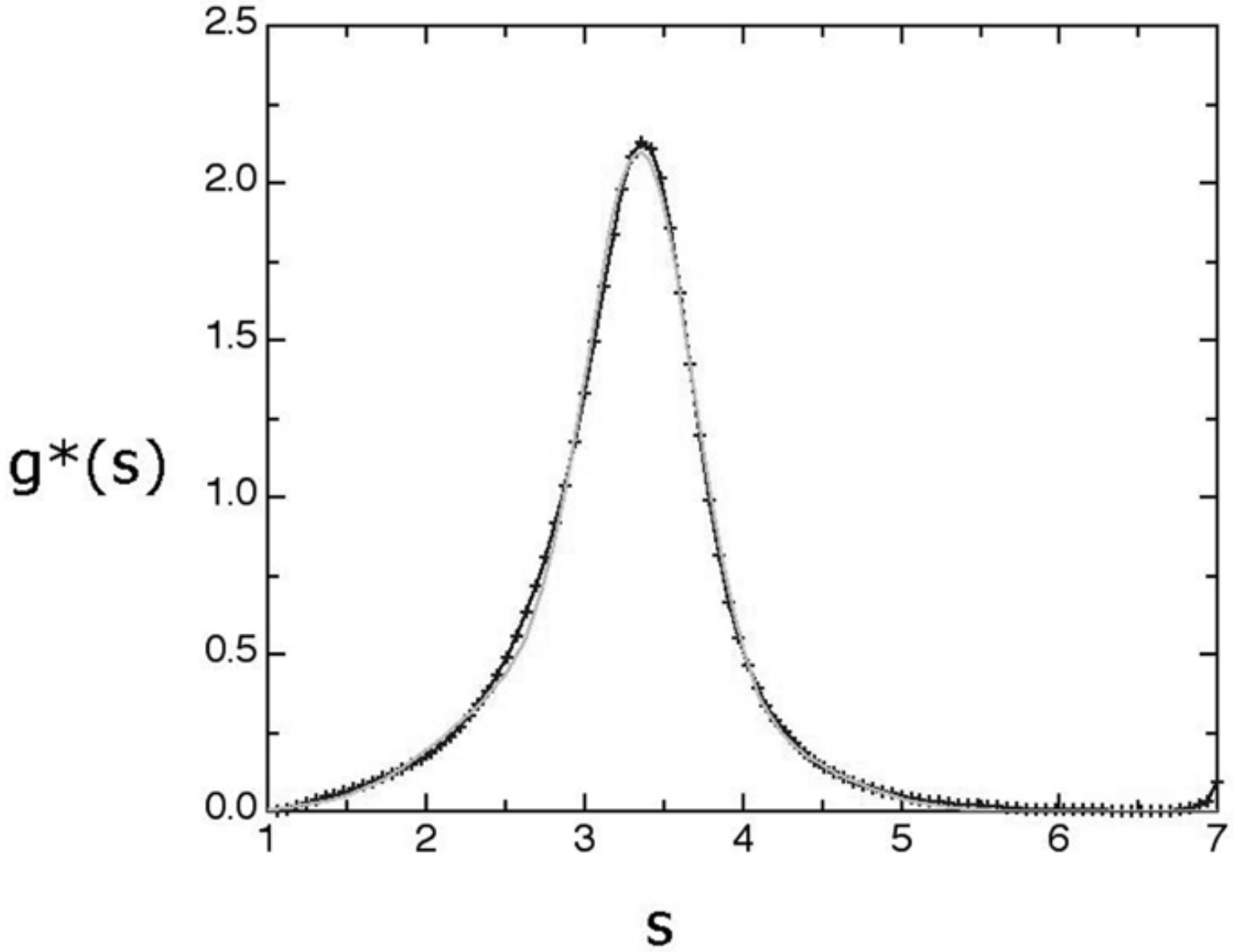
Chitosan G213, 0.5 mg/ml



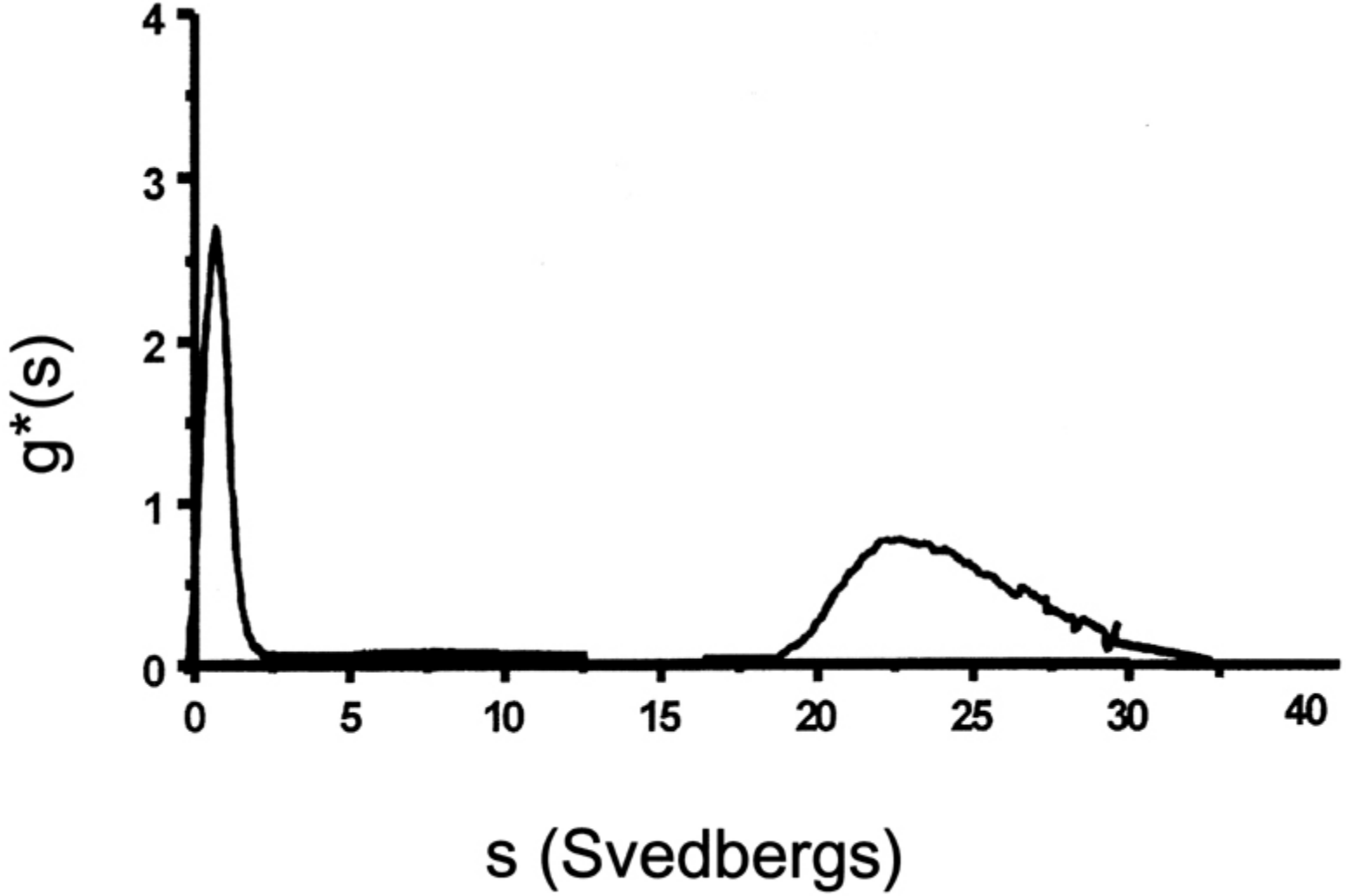
Sedimentation “g(s) plot”:

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

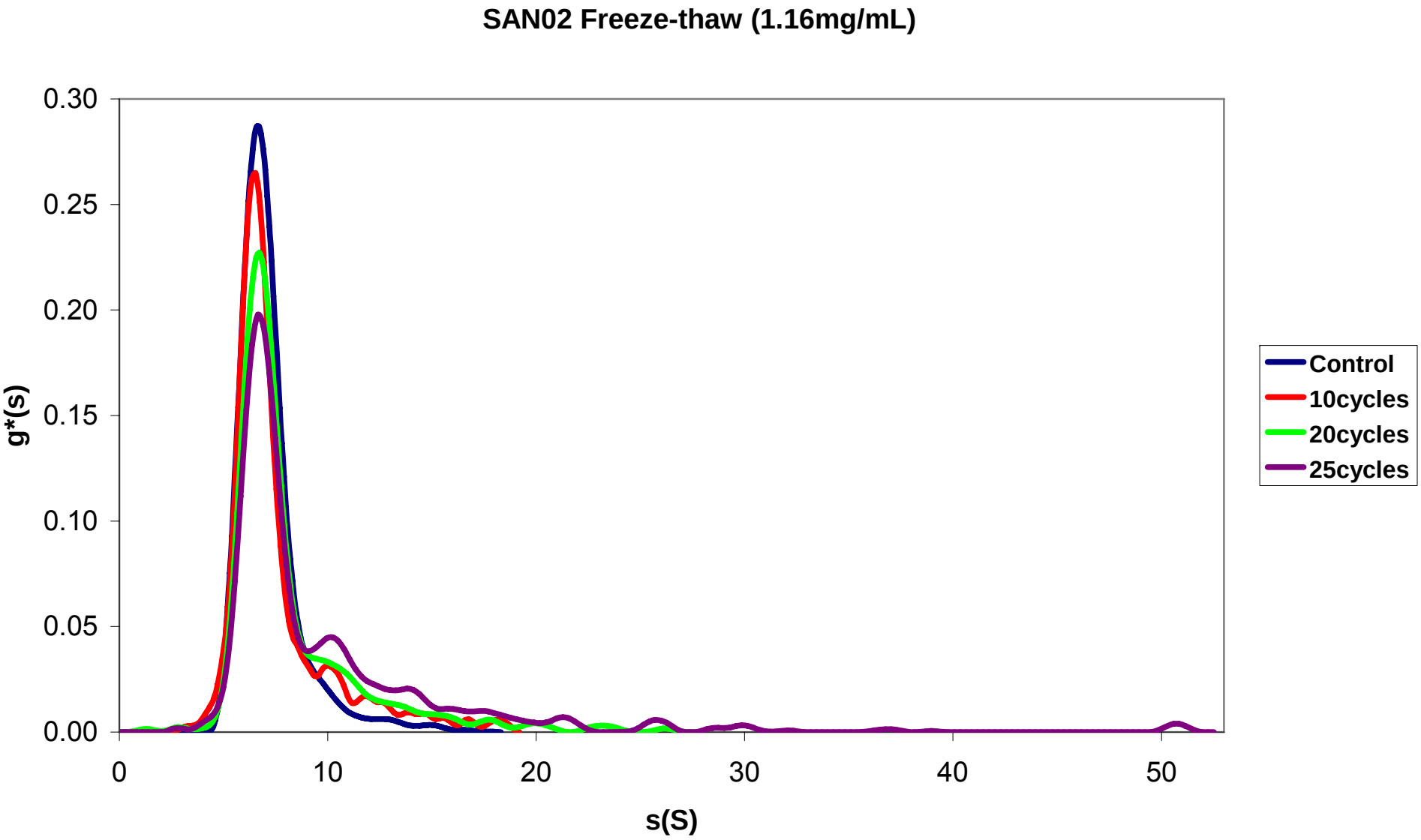
Guar, 0.75 mg/ml



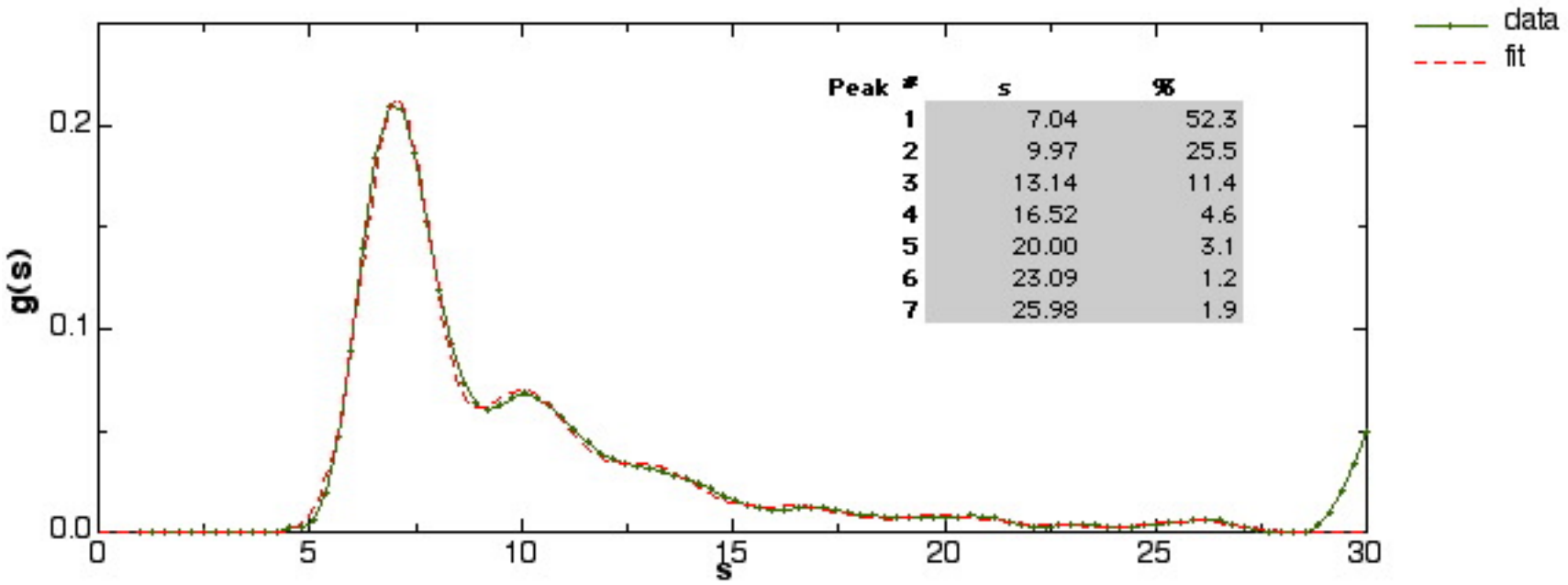
Starch, 8 mg/ml



……stability studies



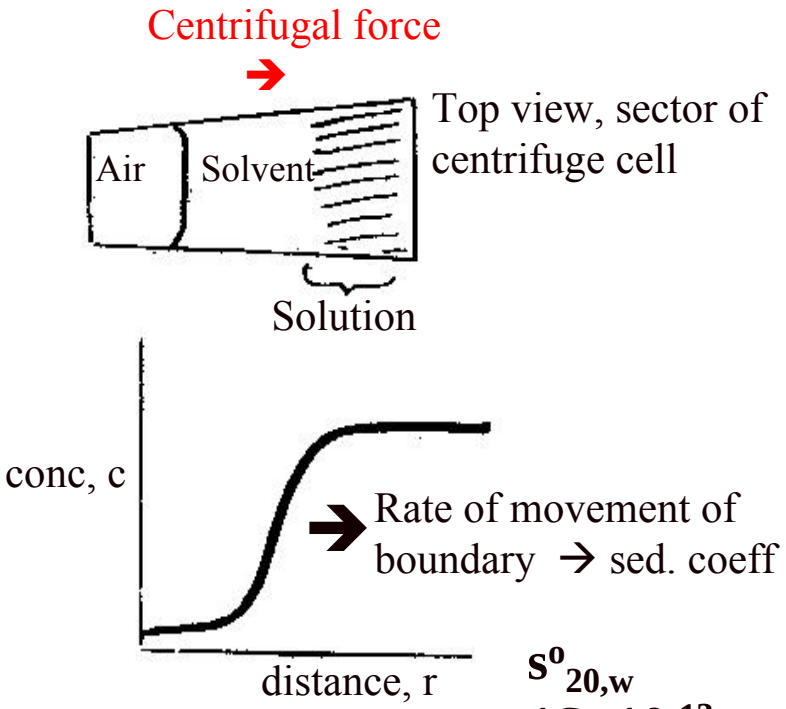
Multi-Gaussian fit estimates *proportions* of each species too:



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

Two types of AUC Experiment:

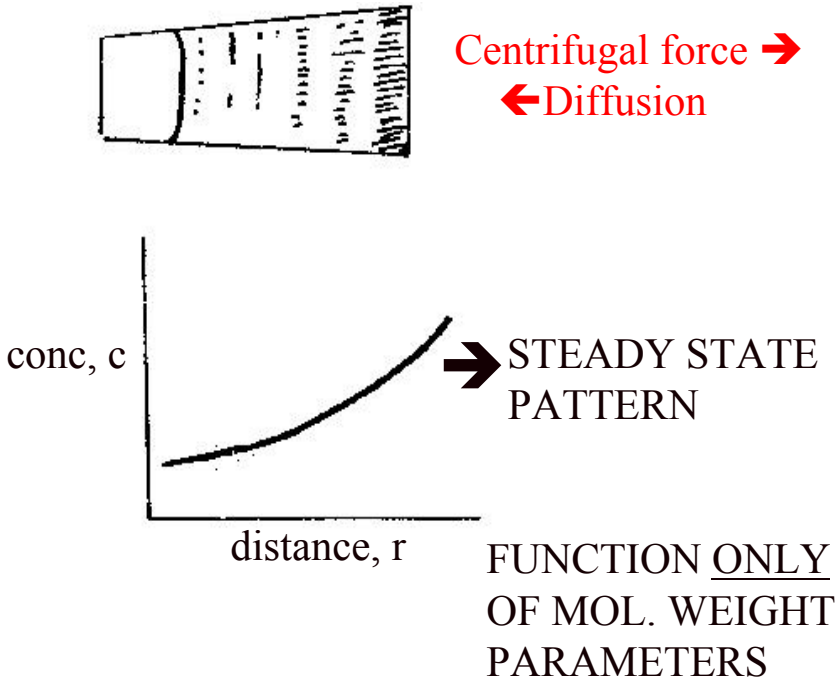
Sedimentation Velocity



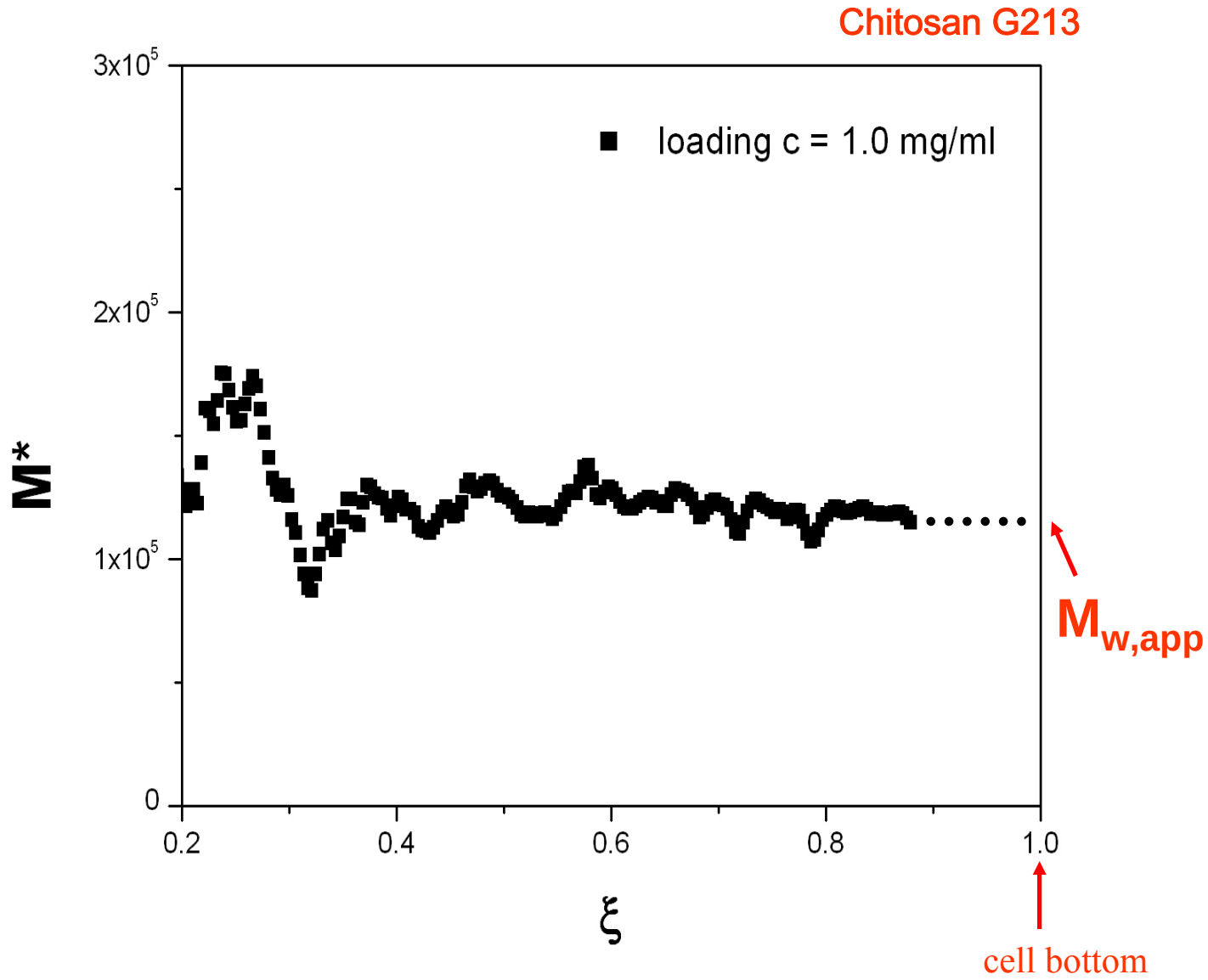
$s^{o}_{20,w}$

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

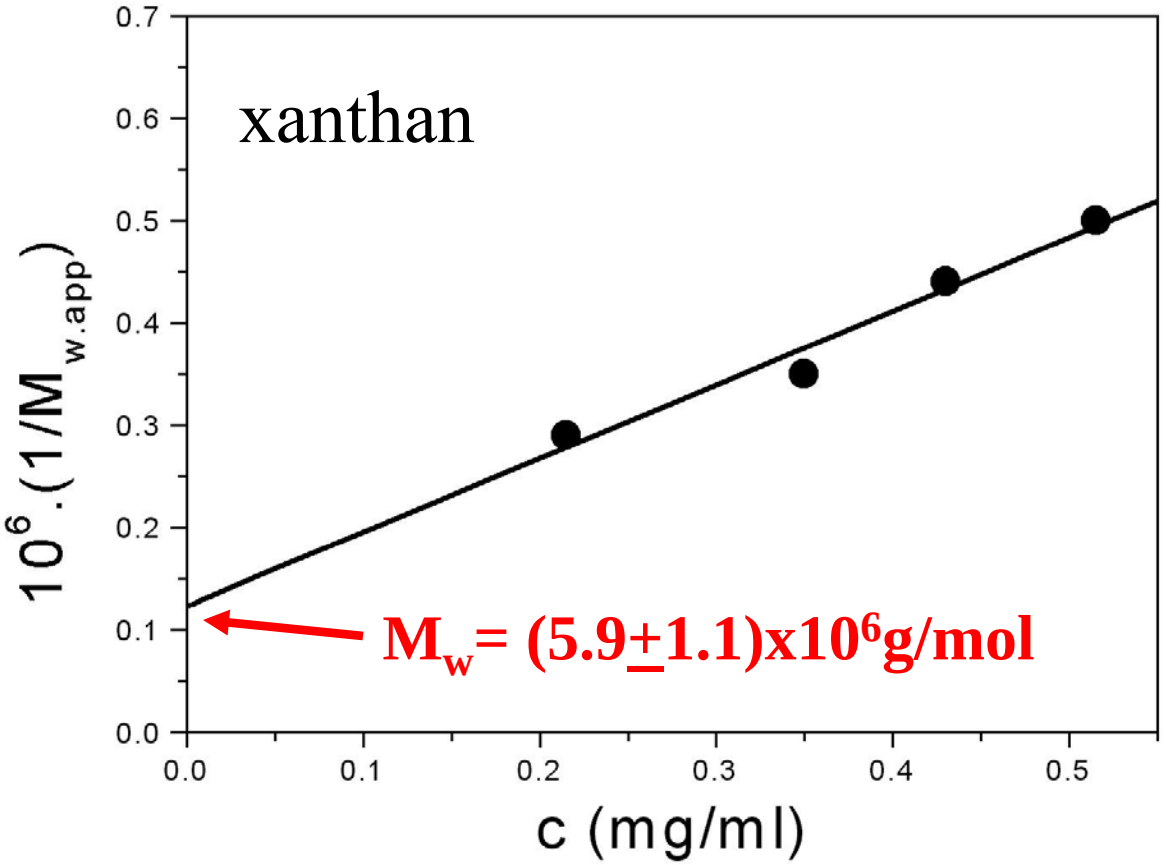
Sedimentation Equilibrium



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



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



Viscometry

$\Rightarrow [\eta]$

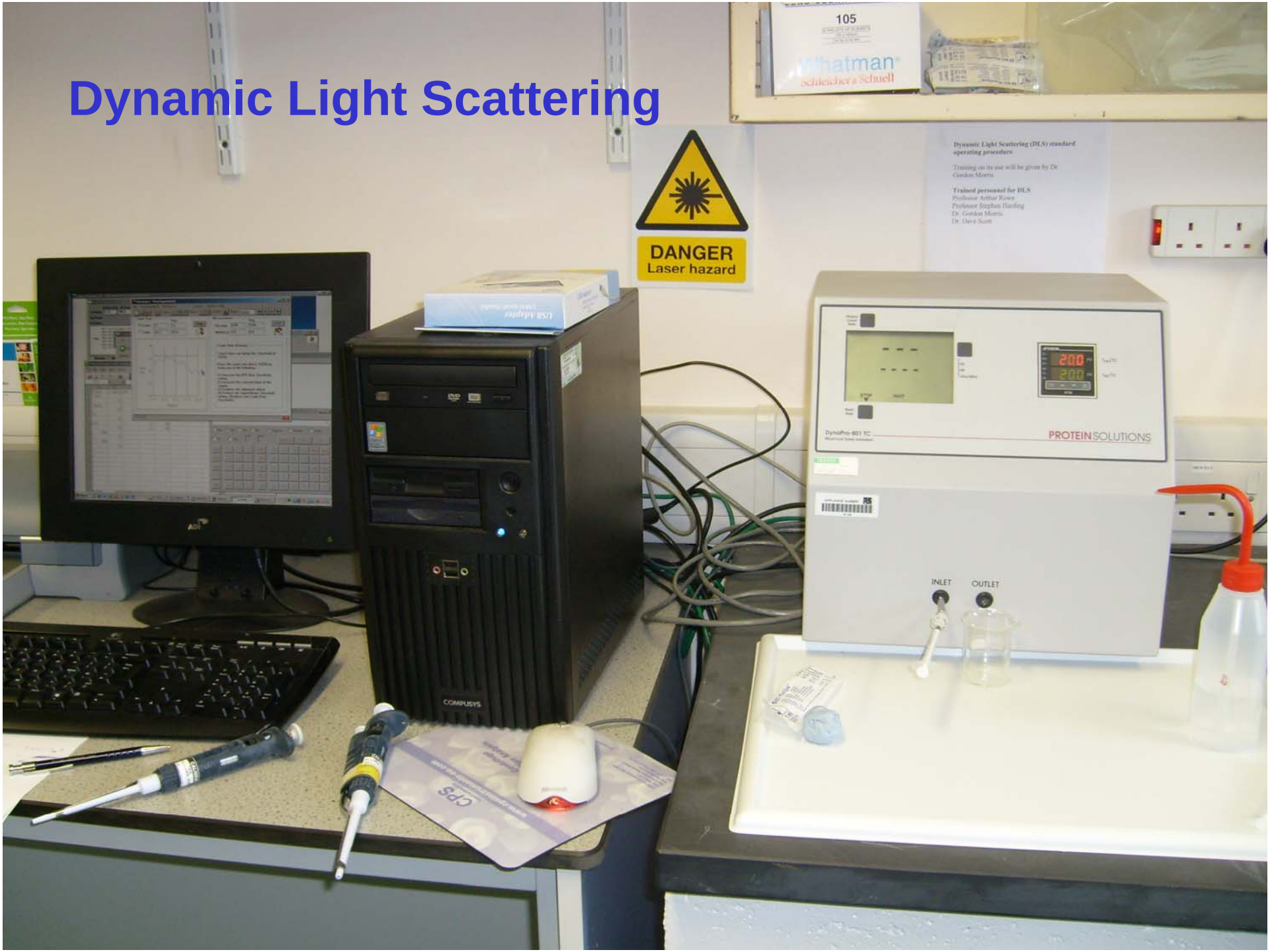
Intrinsic viscosity, ml/g


AMVn
Anton Paar
Automated Micro Viscometer

SEC-MALLs


DAWN
HELEOS-II
Wyatt
ViscoStar-II
viscometer
Wyatt
Optilab
Wyatt

Dynamic Light Scattering



Physical characterisation

1. Heterogeneity: Sedimentation coefficient and distribution. Molecular weight & distribution.
2. Intrinsic Viscosity.. and distribution
3. Conformation in solution, flexibility
4. [Interactions]

Conformation Zoning:

ZONE A

Extra-rigid Rod: Schizophyllan

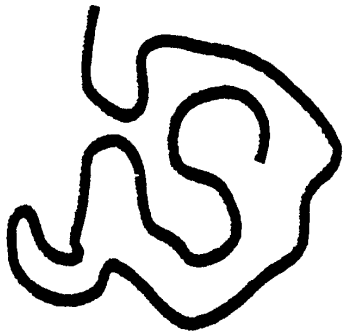
ZONE B

Rigid-rod: DNA

ZONE C

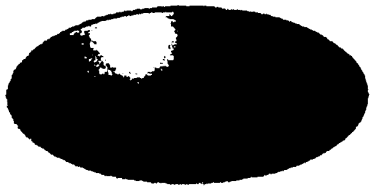
Semi-flexible coil: cellulose derivatives, chitosans

ZONE D



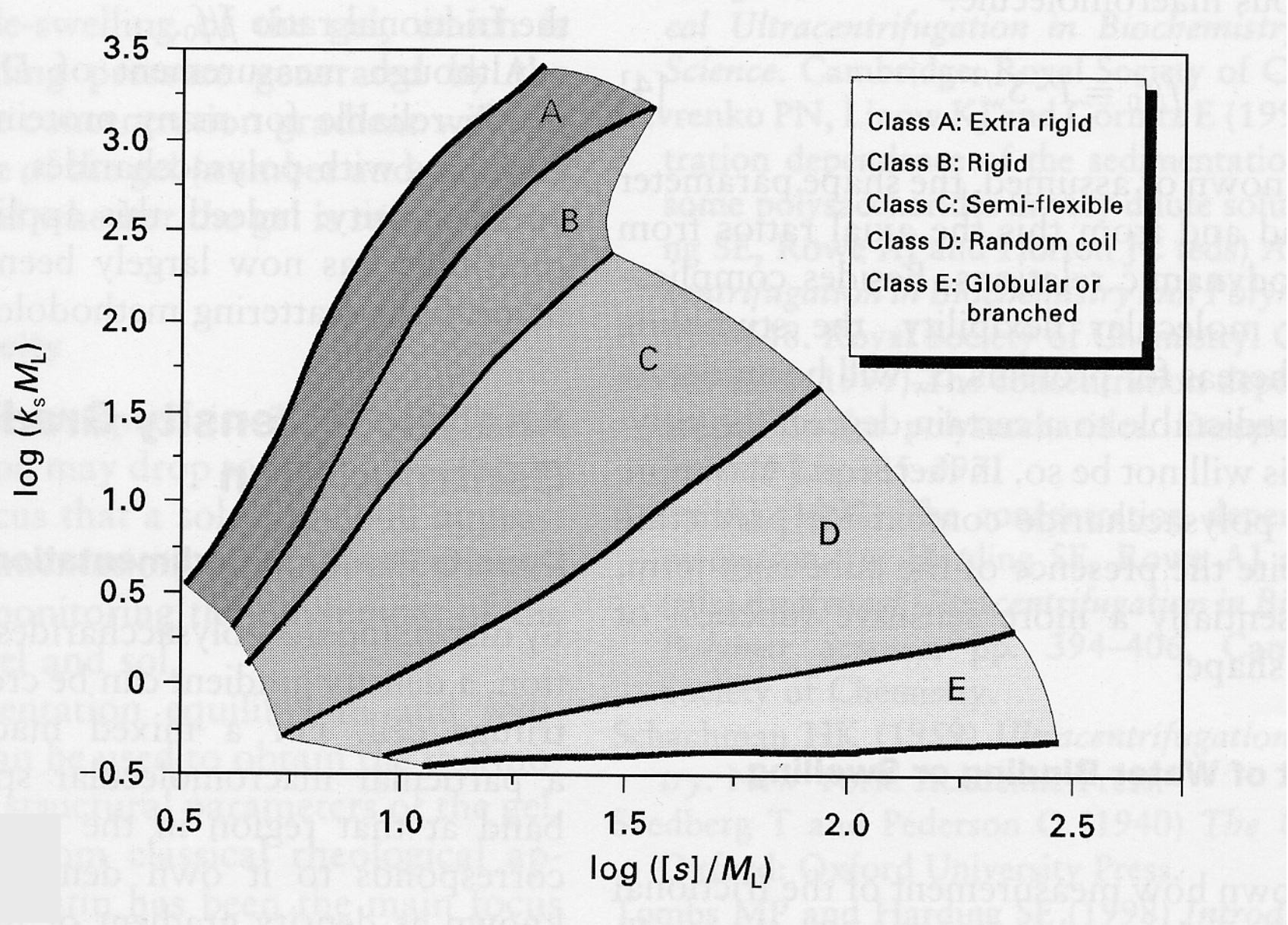
Random coil: Dextran, glycoproteins from mucus

ZONE E

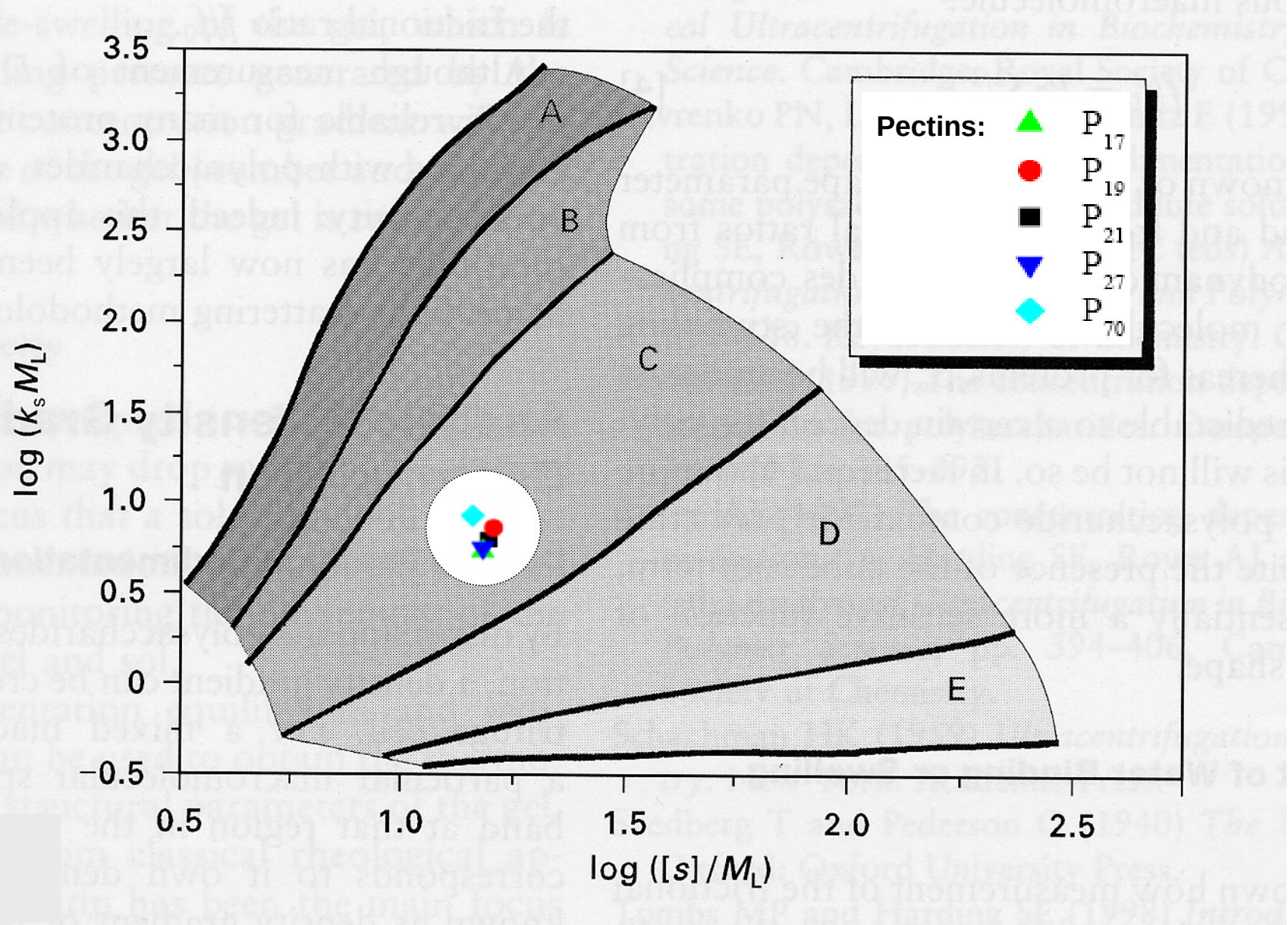


Globular/Branched: Proteins/glycogen

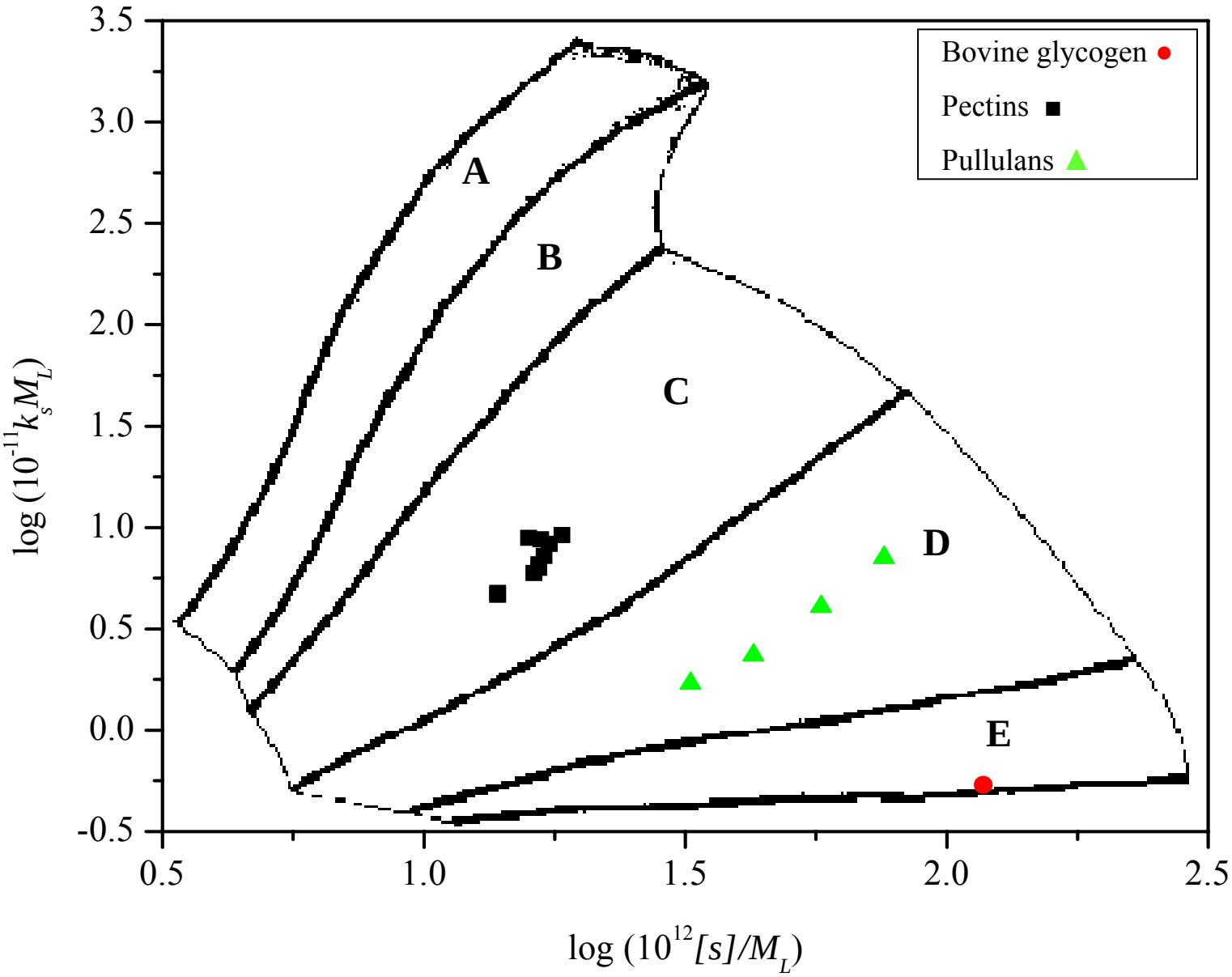
Conformation Zoning:

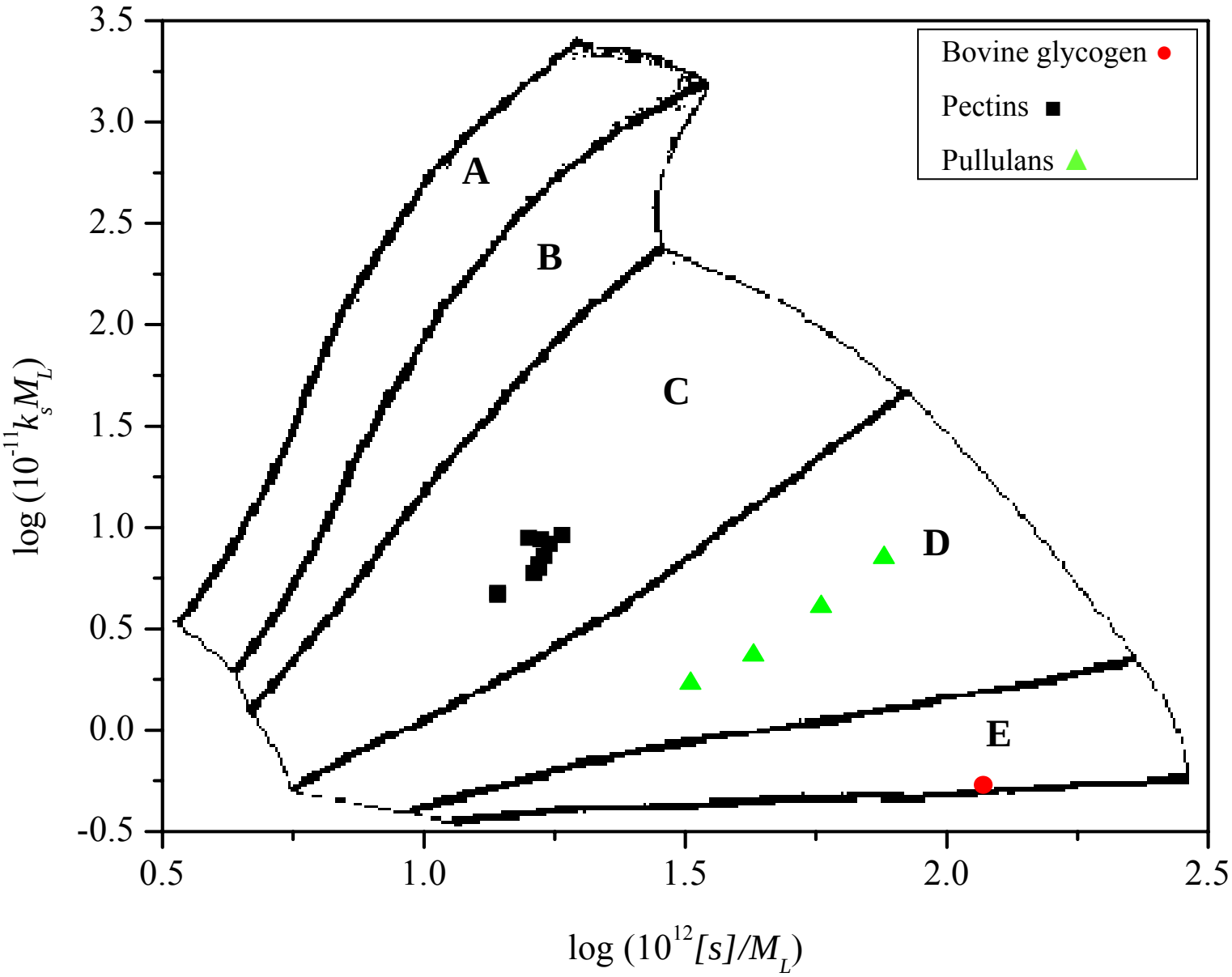


Pavlov, Harding & Rowe., 1997



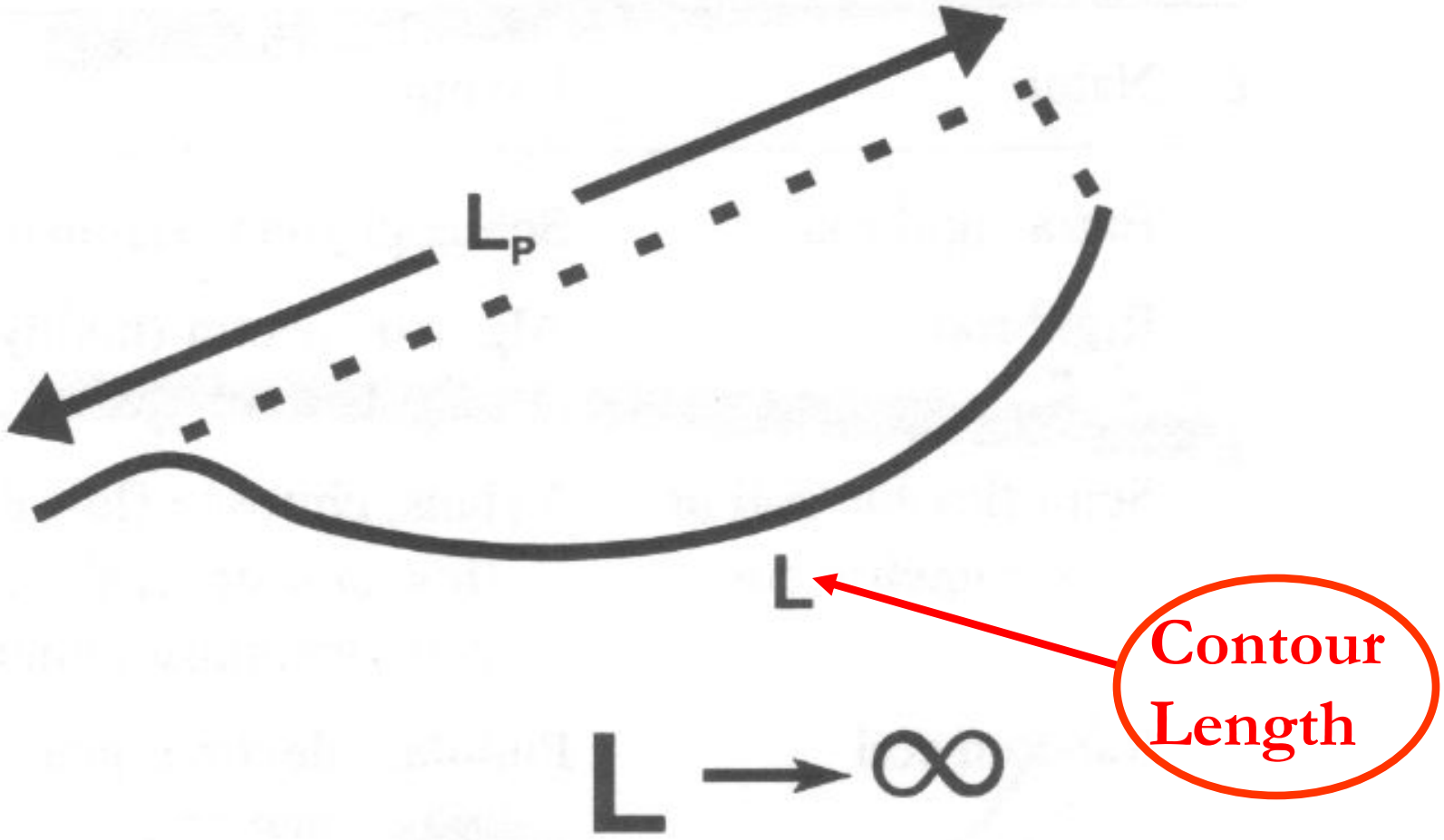
Morris et al., 2007


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)


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

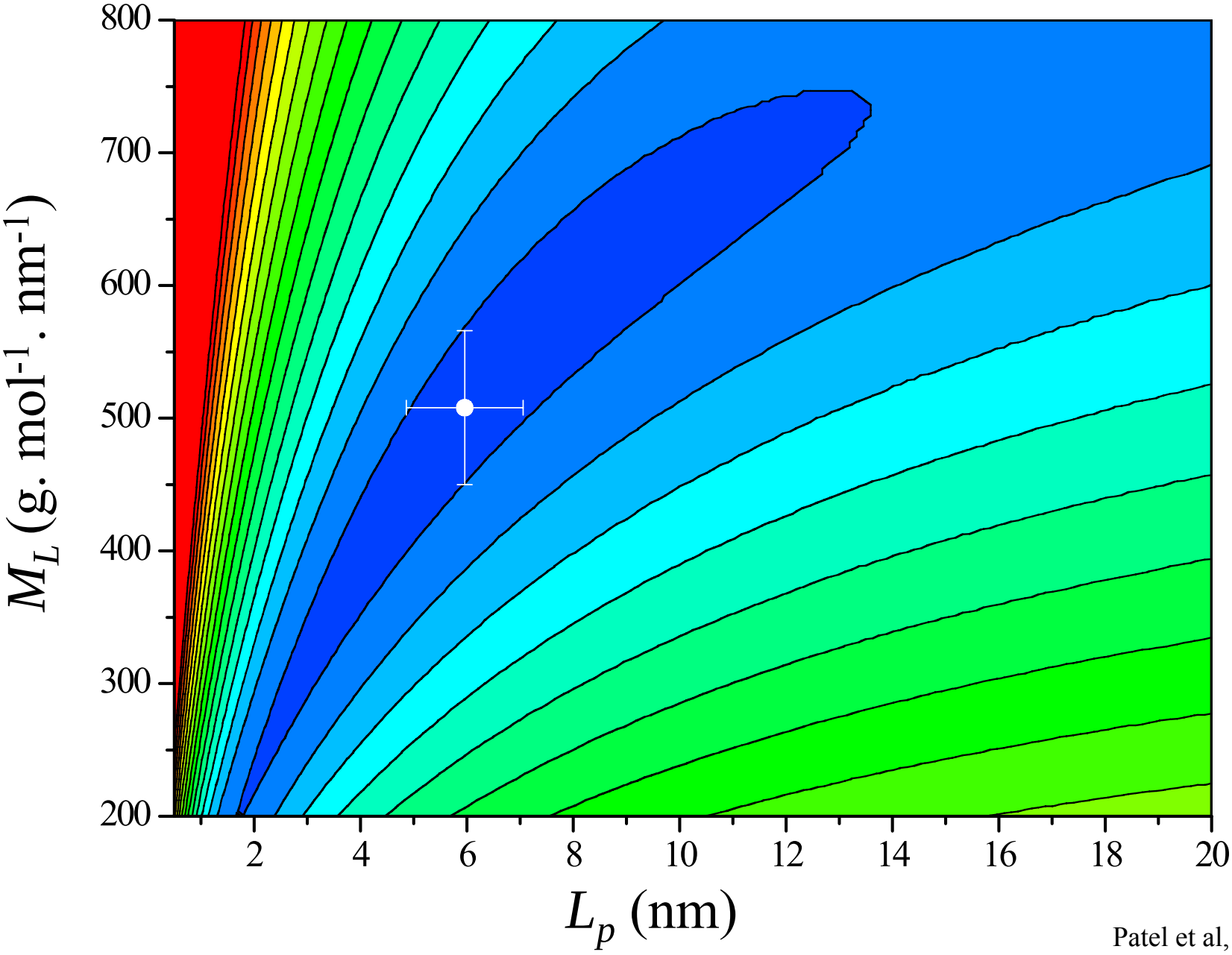
“Bohdanecky” relation

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

“Yamakawa-Fujii” relation

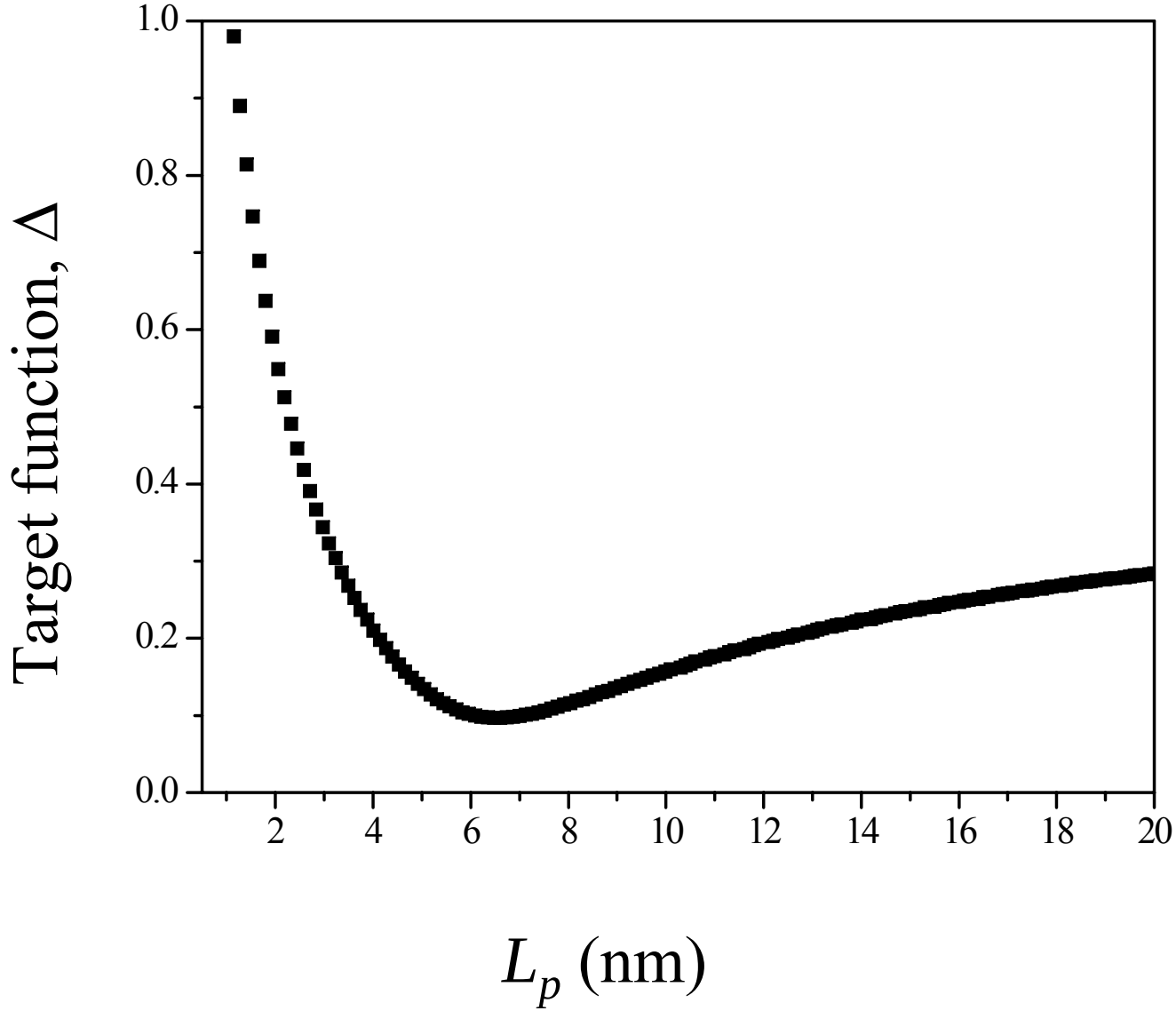
$$s^0 = \frac{M_L\left(1-\bar{v}\rho_0\right)}{3\pi\eta_0 N_A} \times \left[1.843\left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3\left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + \ldots.\right]$$

Global 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

Physical characterisation

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Physical characterisation

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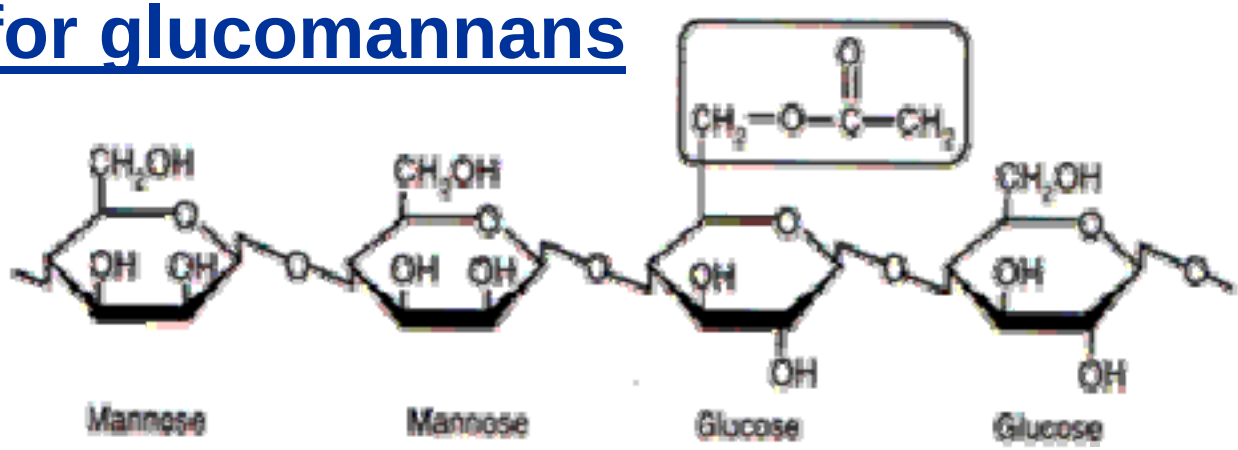
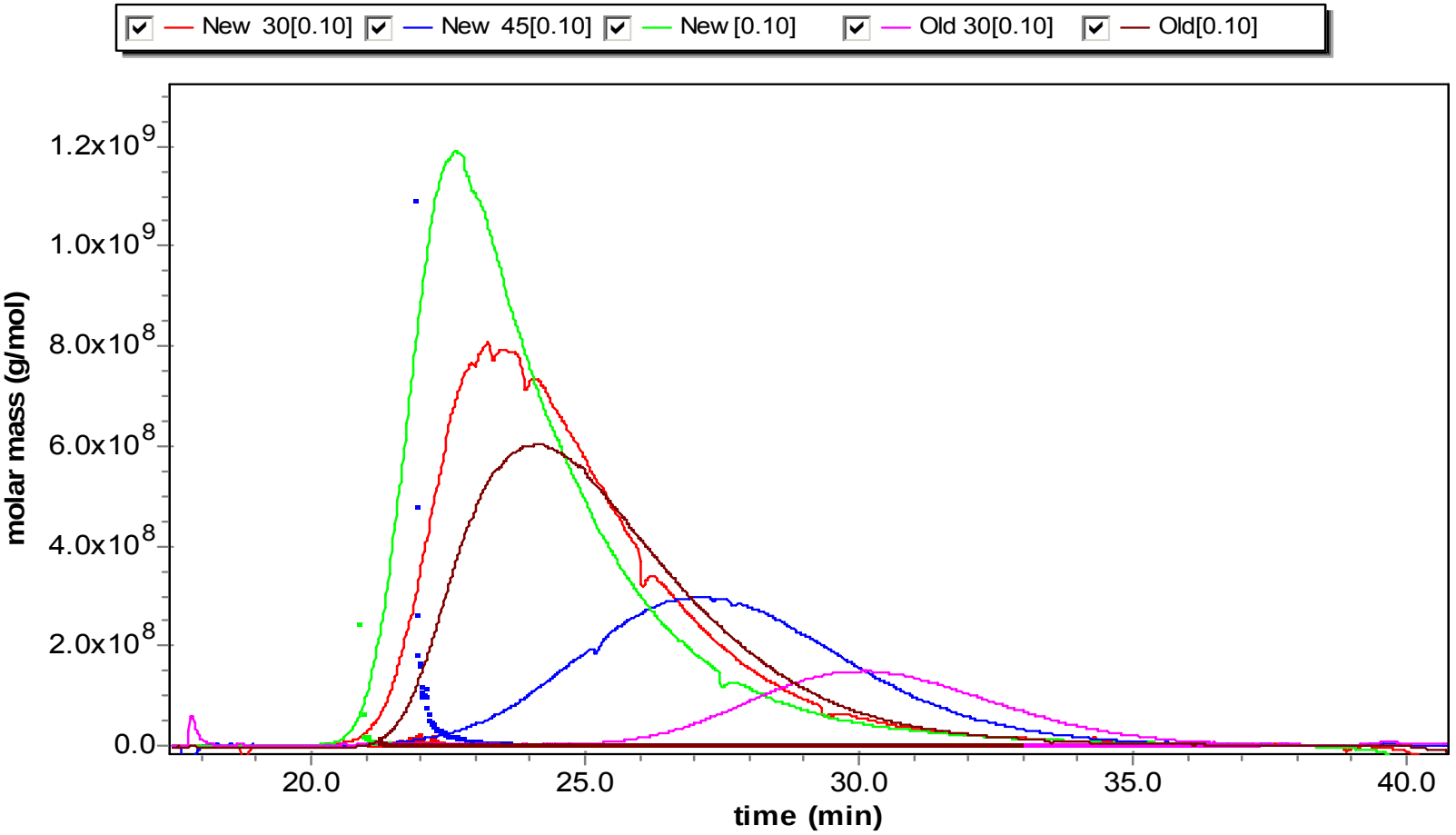
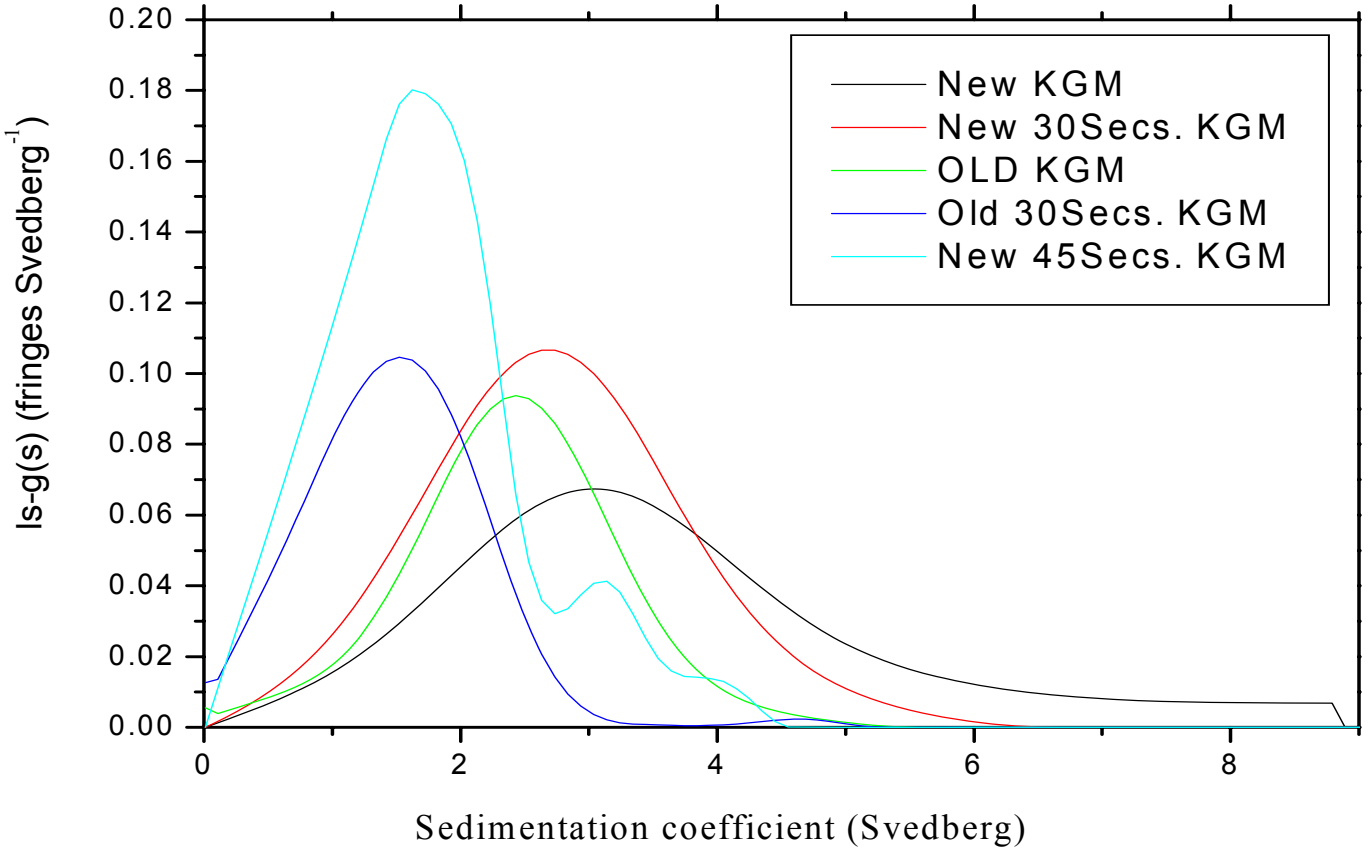
Results for glucomannans



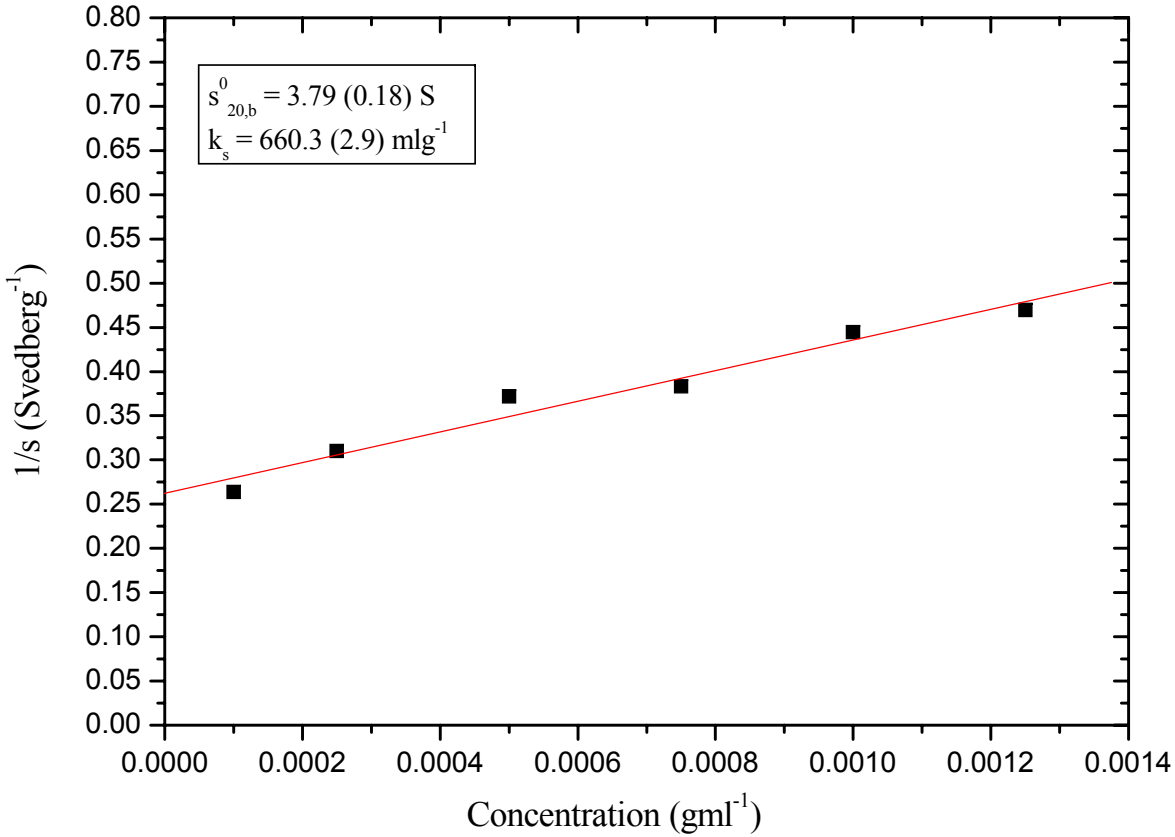
1. Heterogeneity: SEC-MALLs elution profiles



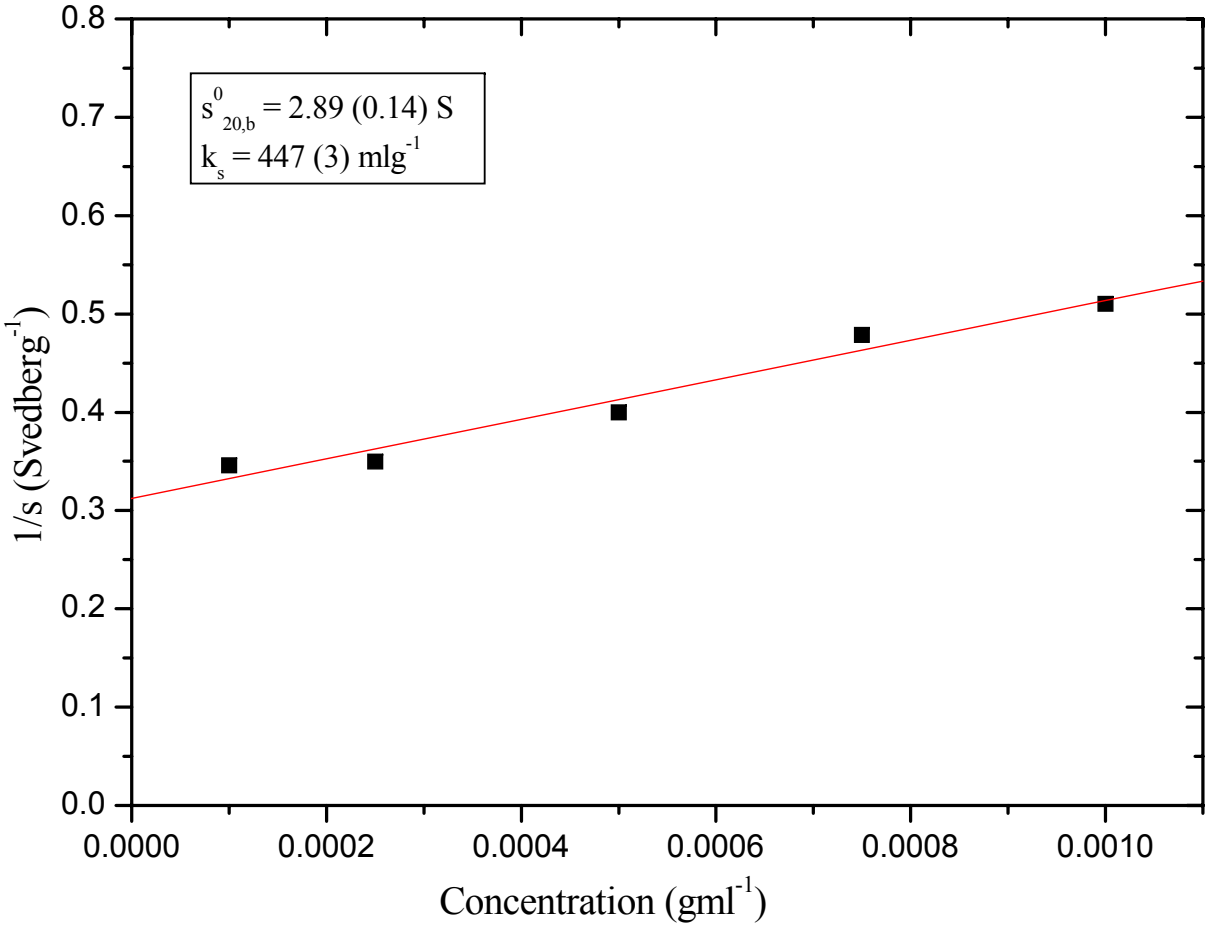
Heterogeneity: Sedimentation coefficient distributions



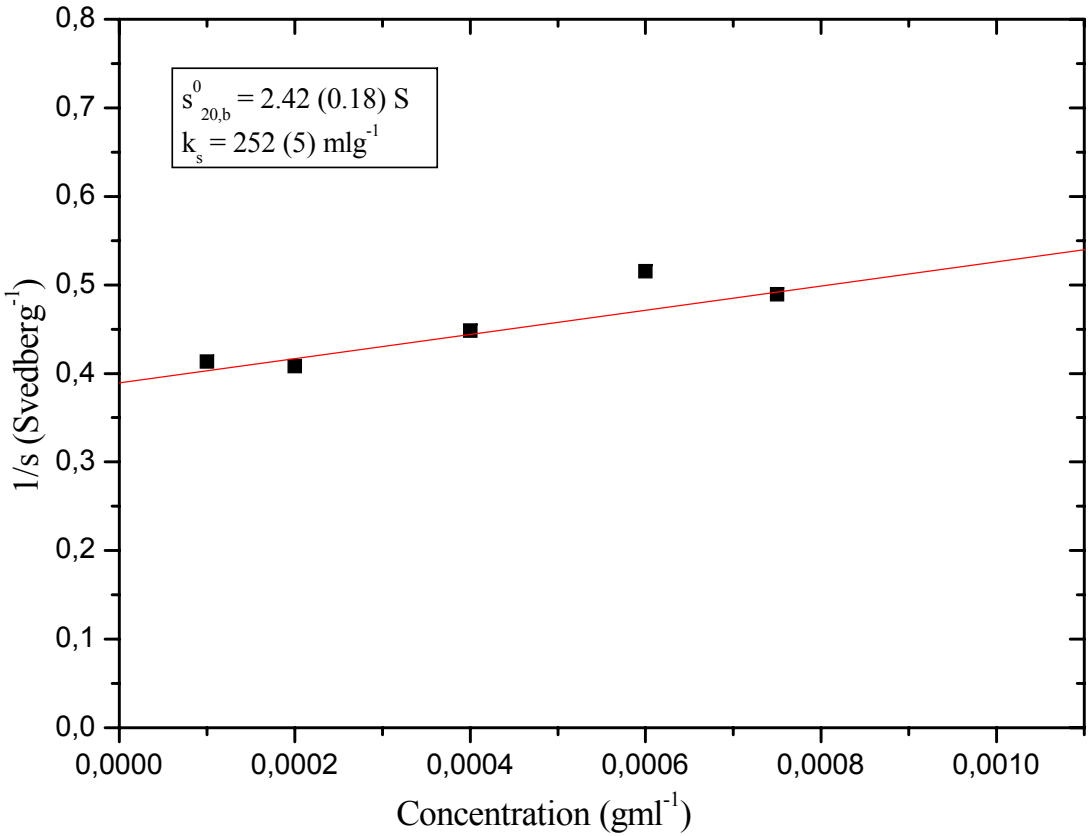
Sedimentation coefficient determination – new KGM



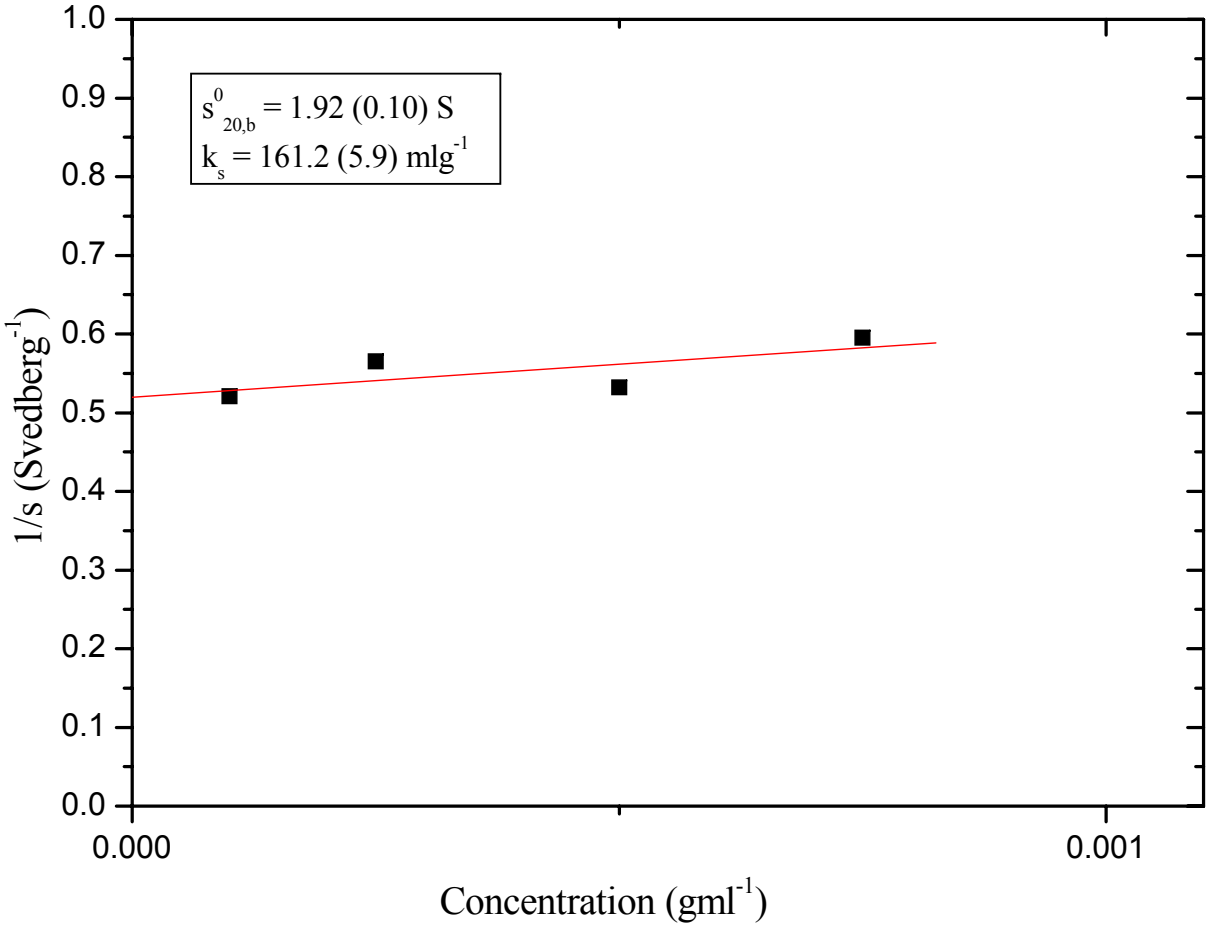
Sedimentation coefficient determination – new KGM processed for 30 seconds



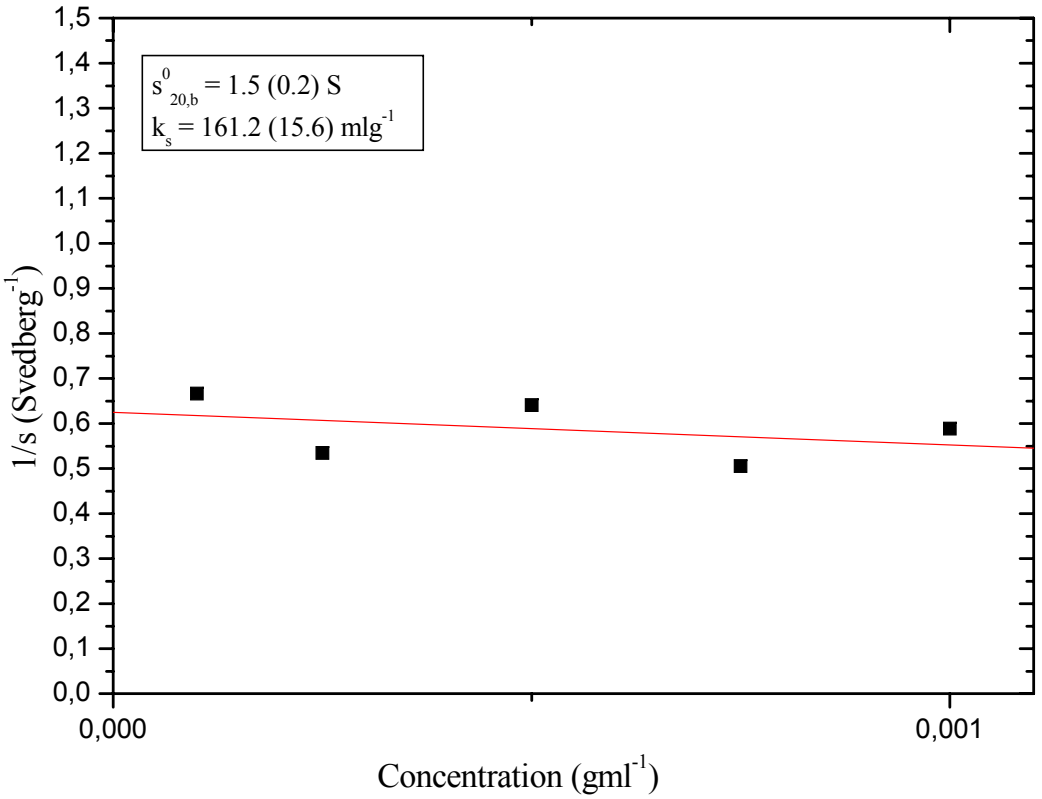
Sedimentation coefficient determination – old KGM



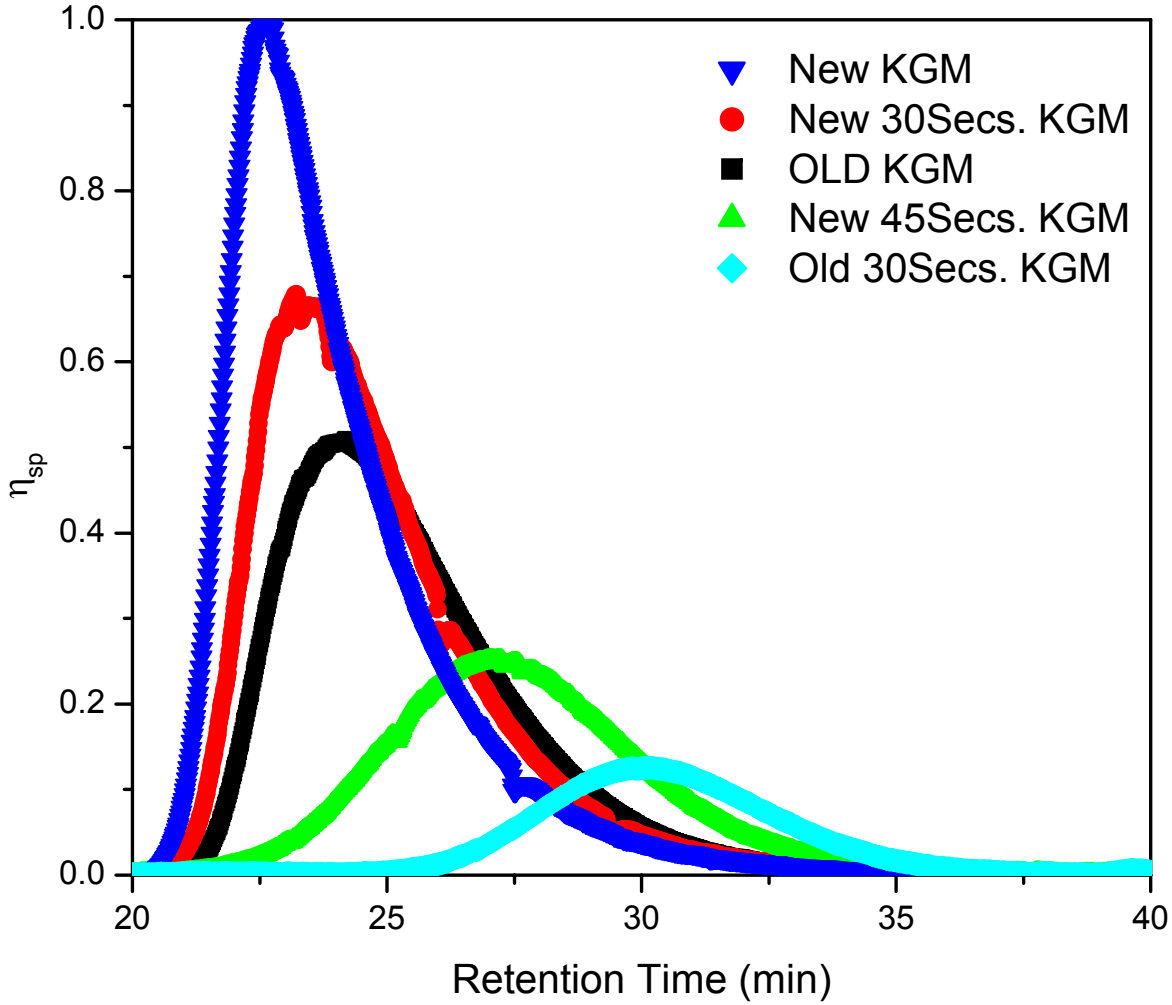
Sedimentation coefficient determination – new KGM processed for 45 seconds



Sedimentation coefficient determination – old KGM processed for 30 seconds



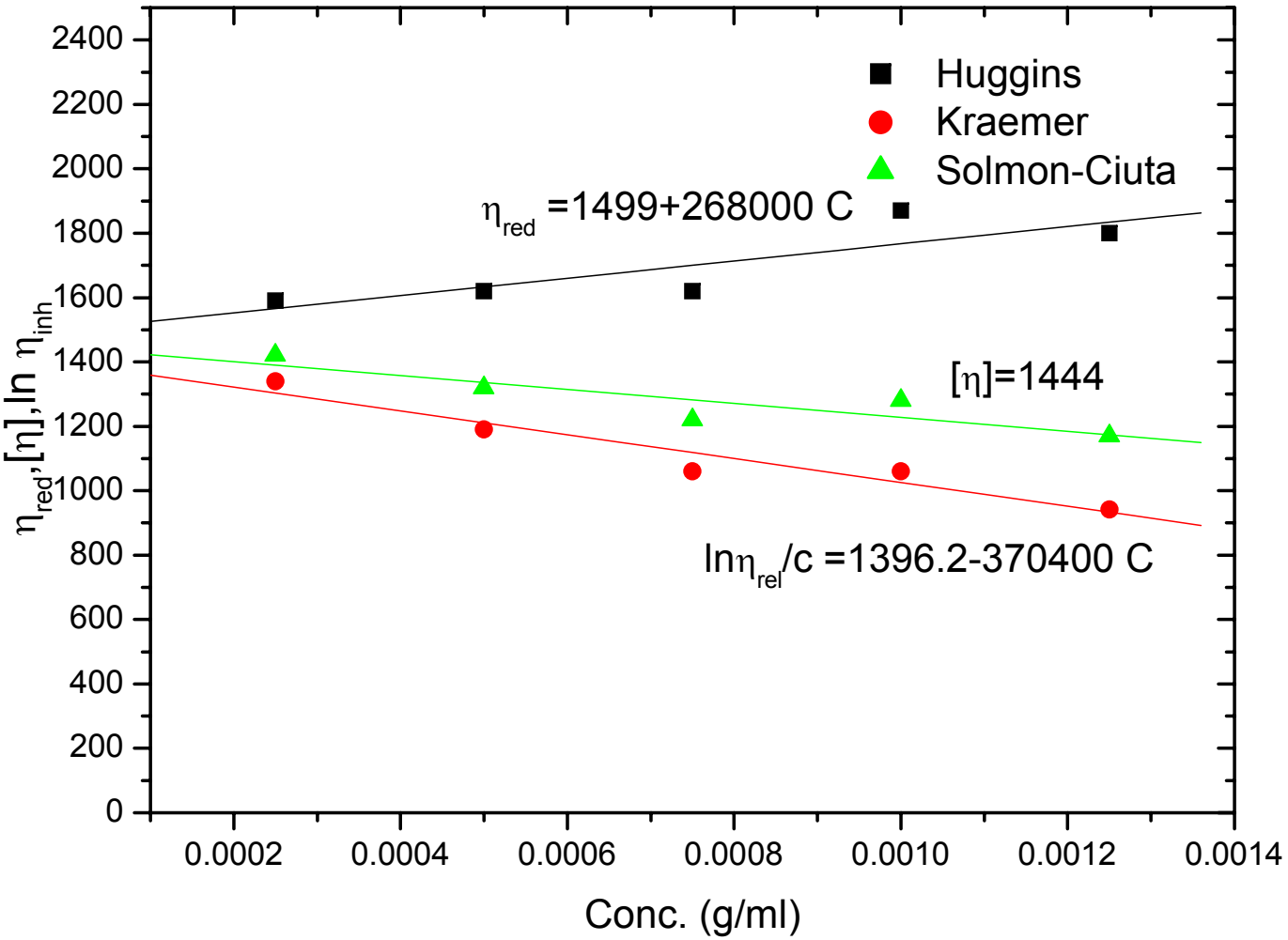
Heterogeneity: specific viscosity distributions (from Viscostar)



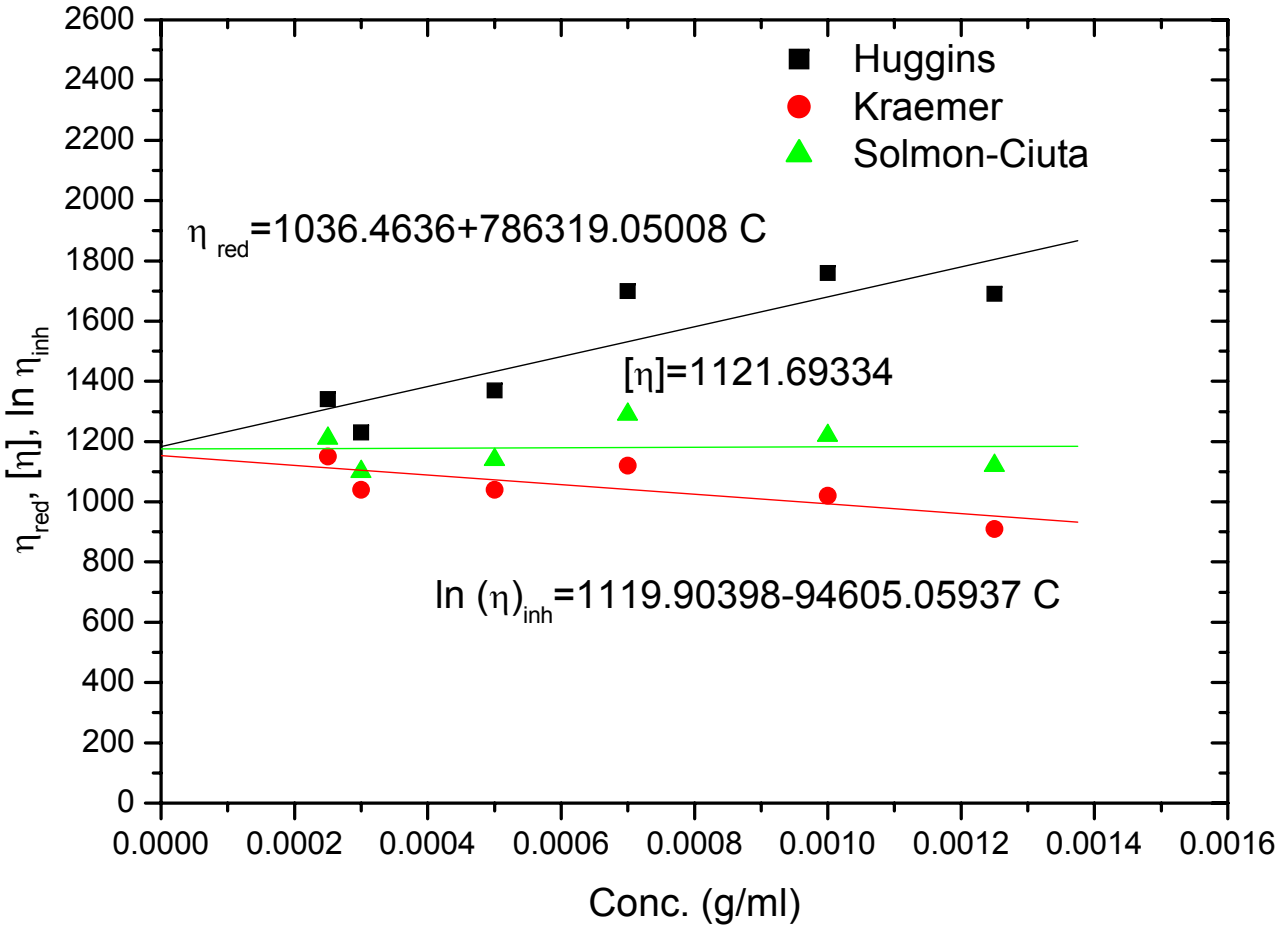
2. Classical intrinsic viscosity measurements from the Anton-Paar viscometer:

The conventional rolling ball technique

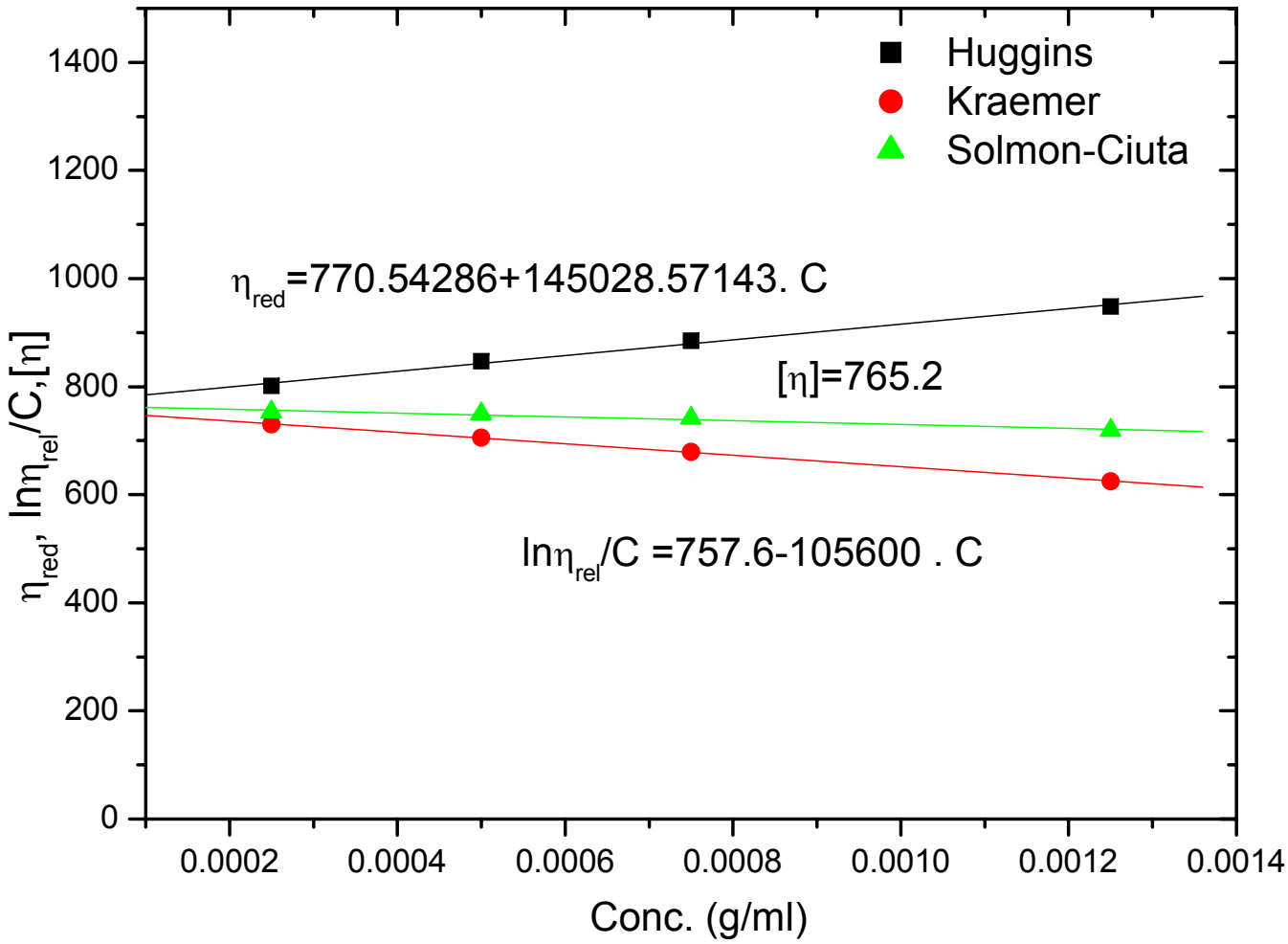
New KGM


Huggins
Kraemer
Solmon-Ciuta
η_{red} =1499+268000 C
[η]=1444
$\ln\eta_{rel}/c$ =1396.2-370400 C
η_{red},[η],ln η_{inh}
Conc. (g/ml)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
0.0002
0.0004
0.0006
0.0008
0.0010
0.0012
0.0014

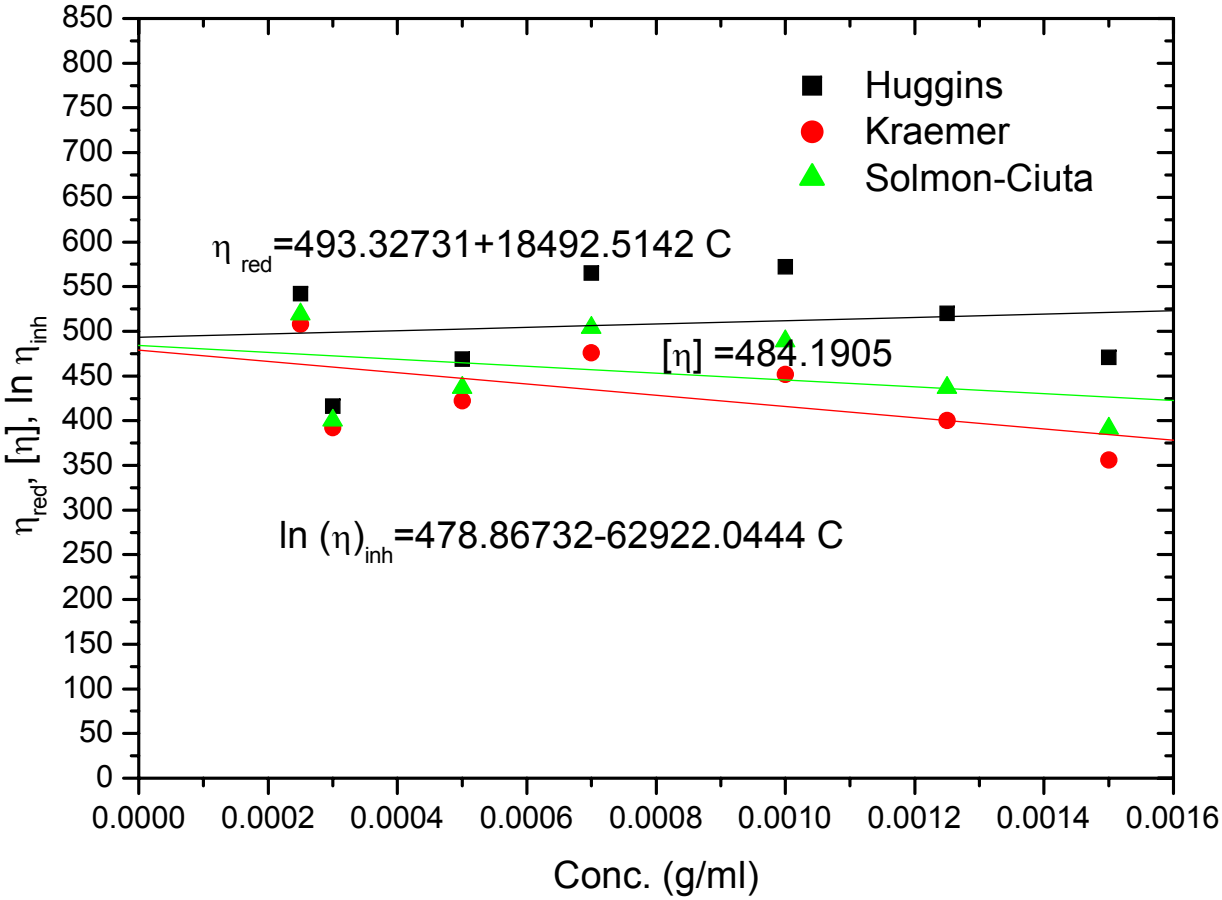
New KGM processed for 30 secs



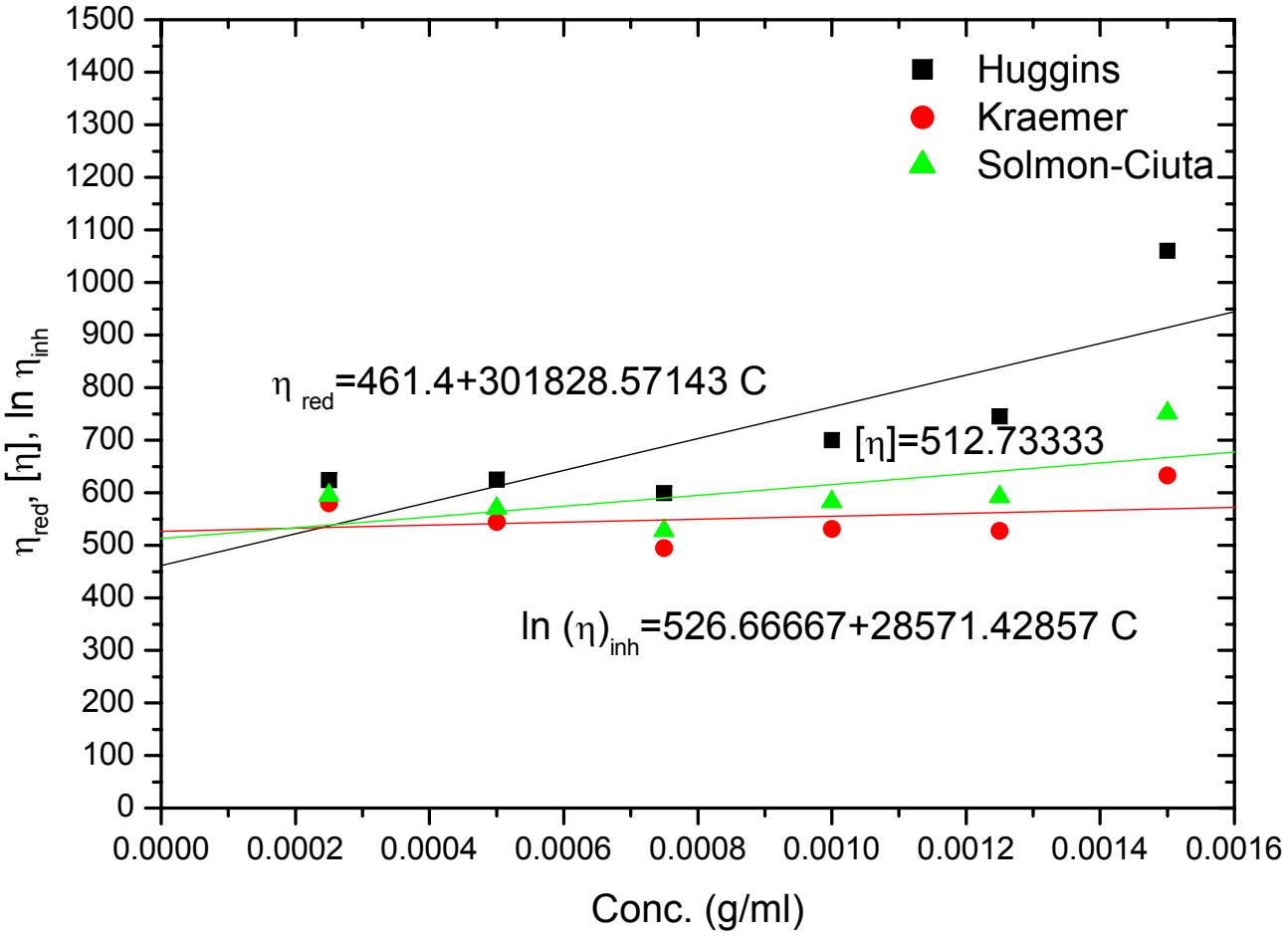
Old KGM


Huggins
Kraemer
Solmon-Ciuta
η_{red}=770.54286+145028.57143. C
[η]=765.2
$\ln\eta_{rel}$/C =757.6-105600 . C
η_{red}, $\ln\eta_{rel}$/C,[η]
Conc. (g/ml)
0
200
400
600
800
1000
1200
1400
0.0002
0.0004
0.0006
0.0008
0.0010
0.0012
0.0014

New KGM processed for 45 secs



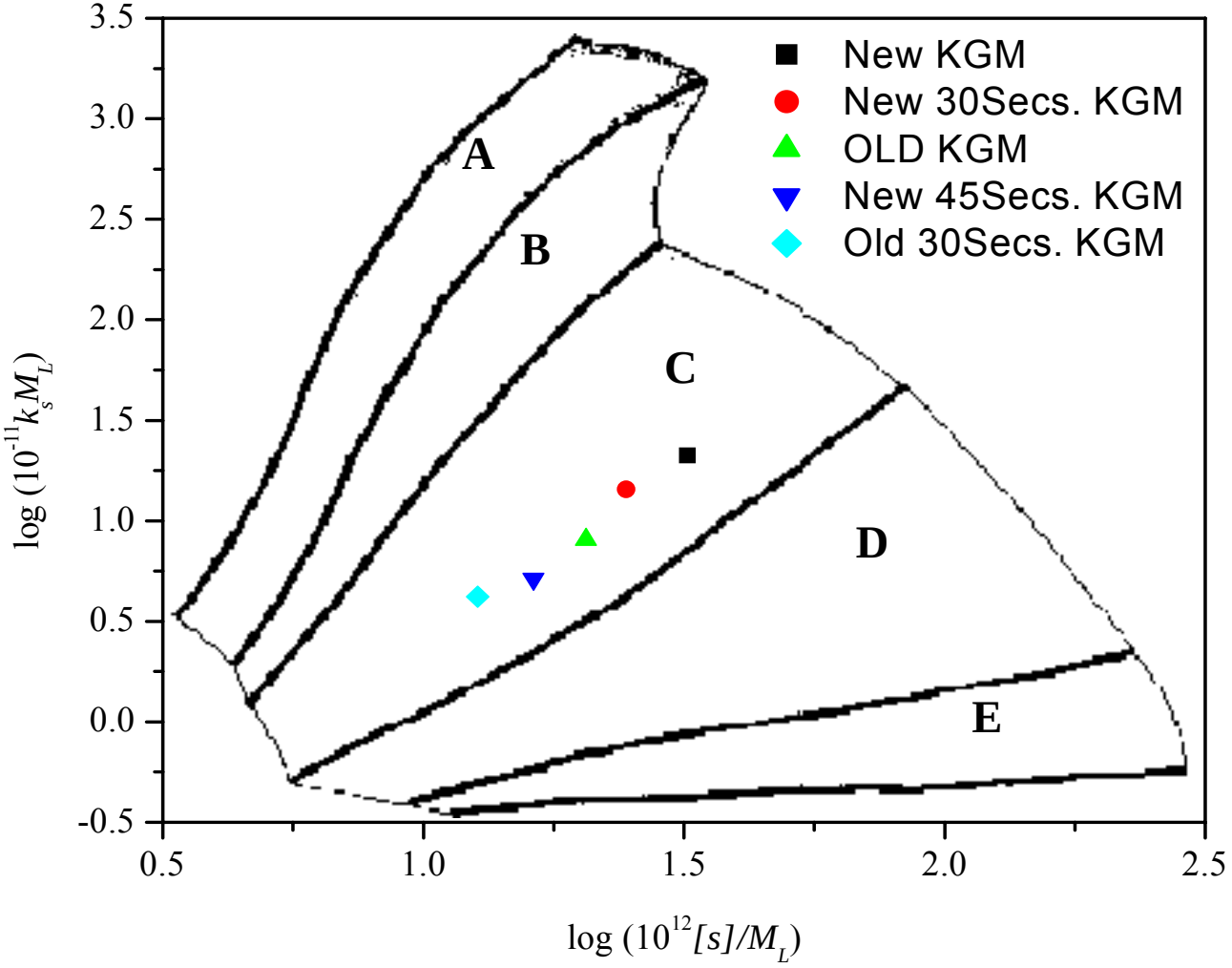
Old KGM processed for 30 secs



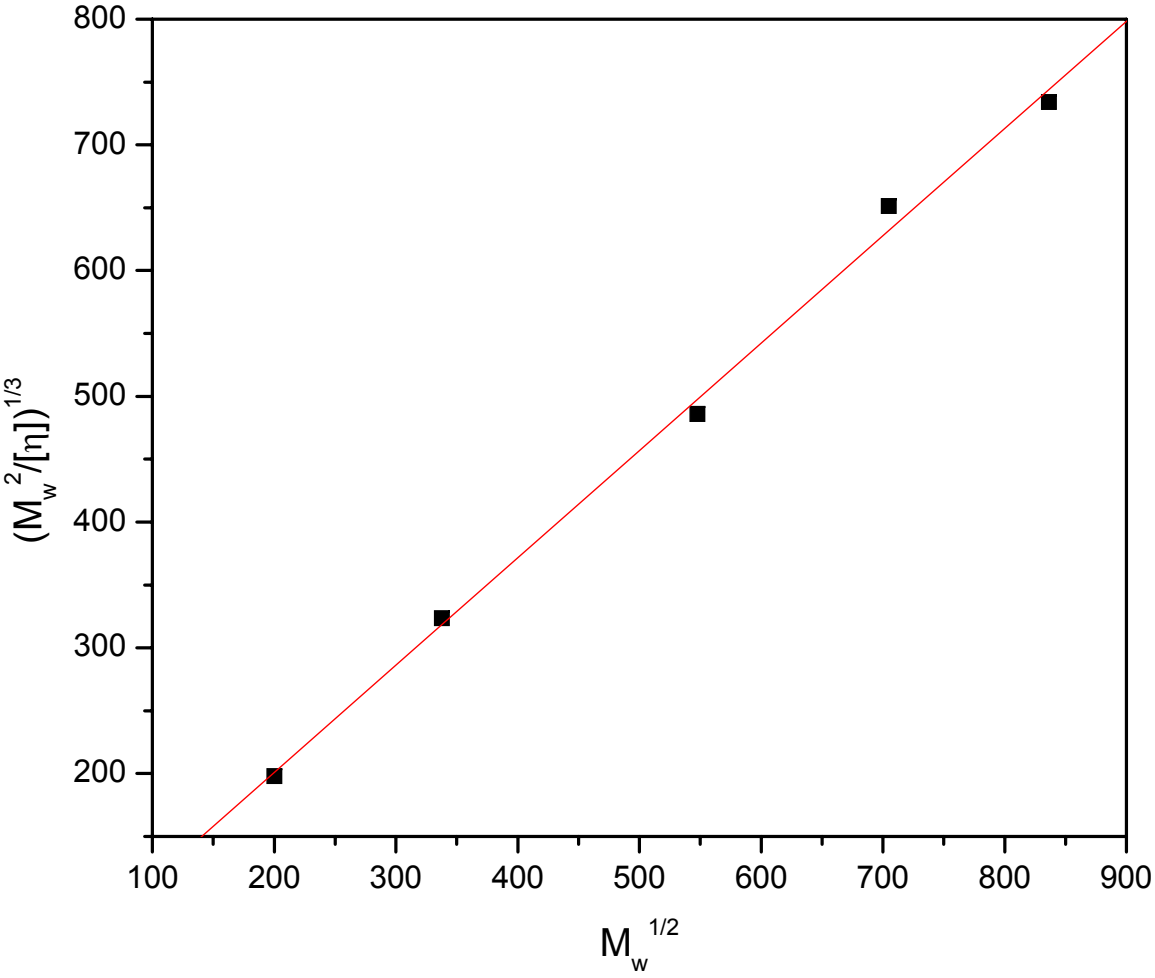
$k_s/[\eta]$ values as an indicator of conformational flexibility. Limits 1.6 (sphere/random coil) and ~ 0.1 for a stiff rod

Sample	$k_s/[\eta]$
New	0.53
New 30 Seconds	0.50
Old	0.32
New 45 Seconds	0.42
Old 30 Seconds	0.63

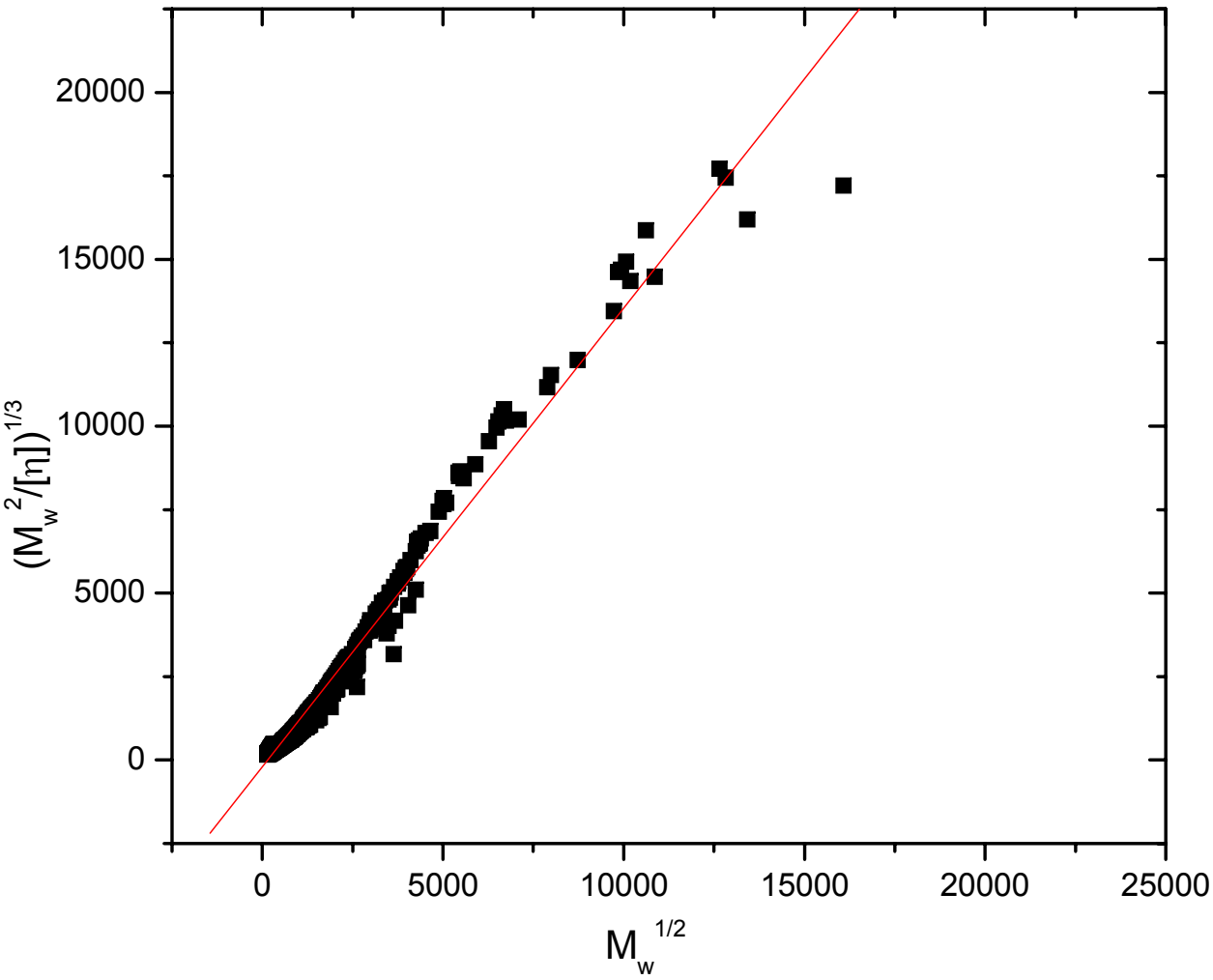
3. Conformation zoning plot – all glucomannans are “Zone C” polysaccharides



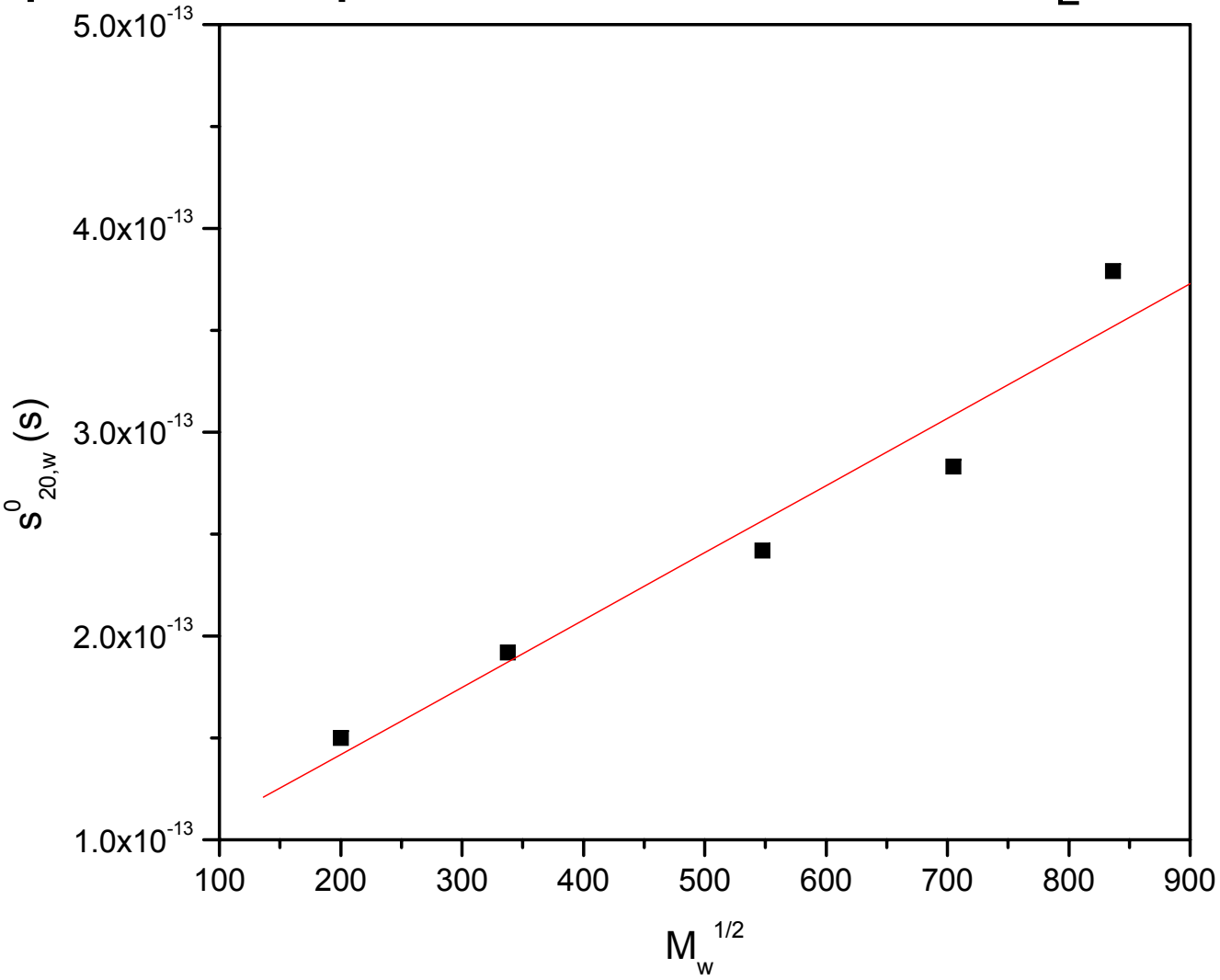
Conformation analysis: Bohdanecky plot. Slope is a measure of M_L and L_p



“On-line” Bohdanecky plot



Conformation analysis: Yamakawa-Fujii plot. Slope is a measure of M_L and L_p



“Bohdanecky” relation

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

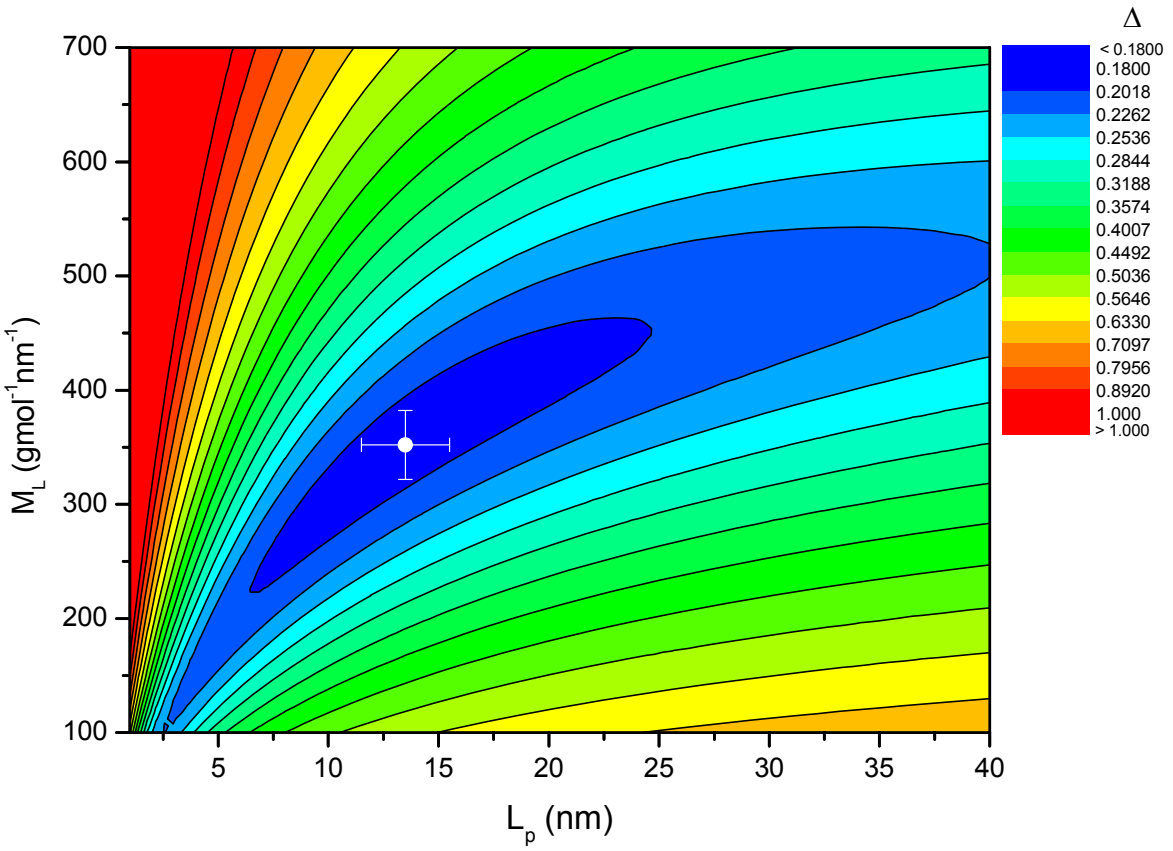
“Yamakawa-Fujii” relation

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

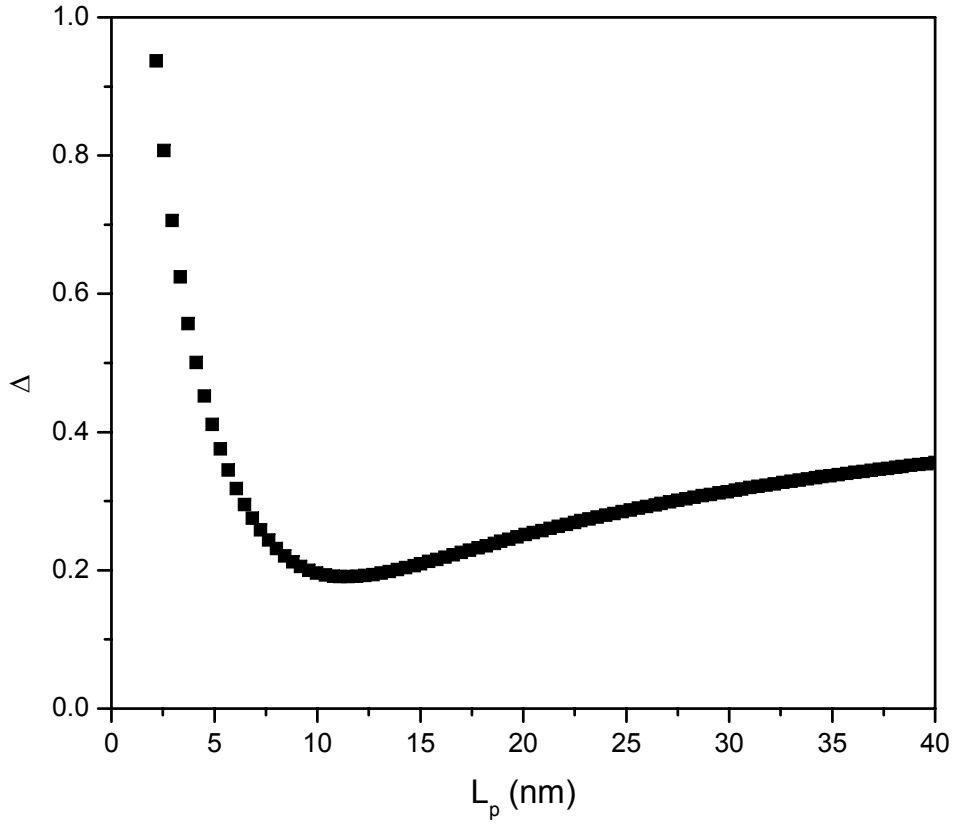
Hydrodynamic data for global analysis

Sample	M_w(g/mol)	[η] (ml/g)	$s^o_{20,w}$ (S)
New	700000	1239	3.79
New 30 Seconds	497000	893	2.89
Old	300000	784	2.42
New 45 Seconds	114000	384	1.92
Old 30 Seconds	40000	208	1.50

Global analysis of glucomannan flexibility



Global analysis of glucomannan flexibility



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for a copy of this presentation …

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