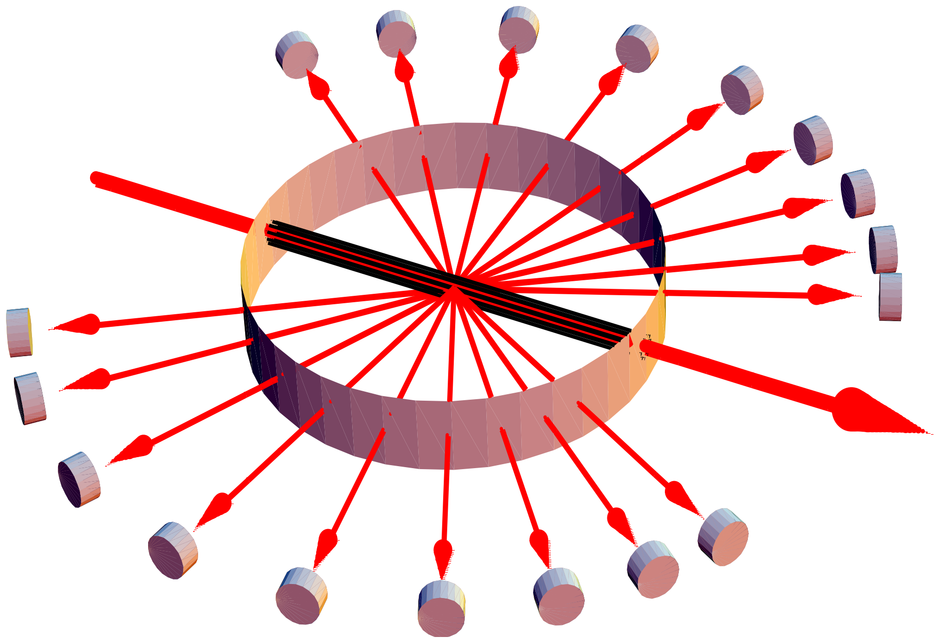
Datum/Zeit	Veranstaltungsort	Thema
Mo, 08.02.2010 10.00-11.30	Hörsaal Institut für Glaschemie Fraunhoferstrasse 6	***Albert Einstein and the Viscosity of Macromolecules***
Mo, 08.02.2010 12.15-13.45	Hörsaal Haus 1,IAAC, August-Bebel-Str. 2	***Light Scattering and SEC-MALLs***
Di, 09.02.2010 12.15-13.45	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 2:

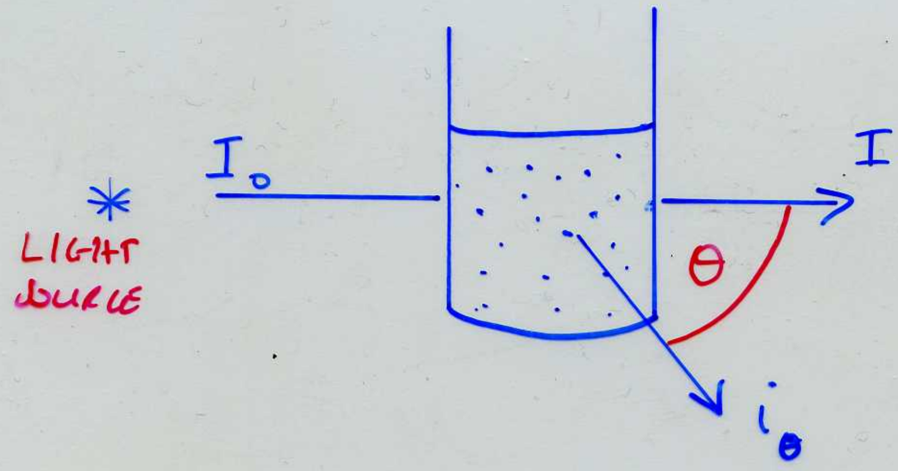
Light Scattering & SEC MALLs



Part 1: Light scattering basic theory (overhead transparencies)

Part 2: Instrumentation, SEC-MALLs and "Triple" Detection

LIGHT SCATTERING



2 MAIN TYPES

① CLASSICAL or "STATIC" LIGHT SCATTERING

- Measure scattered intensity i_θ as a function of angle θ $\longrightarrow$ M_w (weight average molecular weight)
  R_g (radius of gyration)

② DYNAMIC or "QUASI-ELASTIC" LIGHT SCATTERING

- Measure rapid fluctuations of i_θ as a function of time t $\longrightarrow$ D (diffusion coefficient)

CLASSICAL LIGHT SCATTERING

.... for characterisation of biomolecular molecular weights and conformations (via "radius of gyration," R_g)

TYNDALL IN 1864
From a drawing by G. Richmond, R.A., at the Royal Institution

The blue colour of the sky and the polarisation of skylight . . . constitute, in the opinion of our most eminent authorities, the two great standing enigmas of meteorology. Indeed it was the interest manifested in them by Sir John Herschel in a letter of singular speculative power that caused me to enter upon the consideration of these questions so soon.

J. Tyndall, 1969

w2

Historical

17^{th} C	SNELL NEWTON HUYGENS FERMAT	NATURE OF LIGHT; GEOMETRIC OPTICS
19^{th} C	YOUNG FRESNEL	DIFFRACTION / INTERFERENCE

1865 : MAXWELL – ELECTROMAGNETIC THEORY

1869 : TYNDALL – LIGHT SCATTERING ; "THE 2 GREAT STANDING ENIGMAS"

From a drawing by

J.C. MAXWELL

JOHN WILLIAM STRUTT (LORD RAYLEIGH)
In 1870, aged 28, photographed by himself with a wet collodion plate

1881 : RAYLEIGH - SINGLE PARTICLE THEORY {SMALL SCATTERERS}

1890 :	LORENZ	GENERAL THEORY
1908 :	MIE	
:	DEBYE	

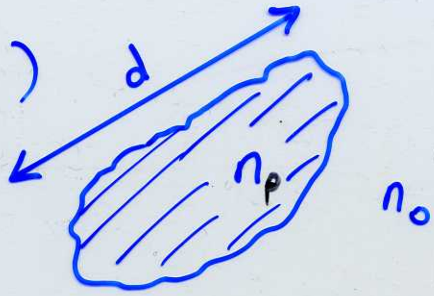
1914 :	RAYLEIGH	APPROXIMATE THEORY
1915 :	DEBYE	
1925 :	GANS	

1908 :	SMOLOCHOWSKI	THERMODYNAMIC THEORY OF SOLUTION SCATTERING
1911 :	EINSTEIN	

1947-50 :	DEBYE	SCATTERING BY SOLUTIONS OF <u>MACROMOLECULES</u>
	ZIMM	

Classification of Light Scattering

(by particle type)



(1) Rayleigh $\quad d \lesssim \frac{\lambda}{20}$ $\quad$ ($M \lesssim 40000$)

- Lysozyme, Myoglobin etc.

(2) Rayleigh - Gans - Debye (RGD)

$$d \sim \lambda/20 \rightarrow \lambda$$

$$\frac{n_p}{n_o} - 1 \ll 1$$

OF MOST INTEREST!

(3) Mie $\quad d \gtrsim \lambda$

LARGE VIRUSES, BACTERIA etc.

RAYLEIGH - GANS - DEBYE (RGD) SCATTERING.

- for biomolecules of $M \simeq 40000 \rightarrow 20 \times 10^6$

MEASURE A PARAMETER 'RAYLEIGH RATIO'

$$R_\theta = \frac{i_\theta}{I_0} \cdot \left[\frac{r^2}{1 + \cos^2\theta} \right]$$

(r: dist. of particle from detector)

LIGHT SCATTERING BY A SOLUTION OF MACROMOLECULES CAN BE SUMMARISED BY THE EQUATION:

$$\frac{KC}{R_\theta} \simeq \left\{ 1 + \frac{16\pi^2 R_g^2}{3\lambda^2} \sin^2\frac{\theta}{2} \right\} \left(\frac{1}{M} + 2BC \right)$$

K is a collection of constants : $\dfrac{2\pi^2 n_0^2 (dn/dC)^2}{N_A \lambda^4}$

B : 2^{nd} virial coeff. ; C : concentration

R_G : "radius of gyration".

If B is known, or C is small enough $(2BC \simeq 0)$

a plot of $\dfrac{KC}{R_\theta}$ vs $\sin^2\dfrac{\theta}{2}$ $\rightarrow$ Mol. wt & R_g.

ZIMM PLOT : ALGINATE POLYSACCHARIDE (M ~ 200,000)

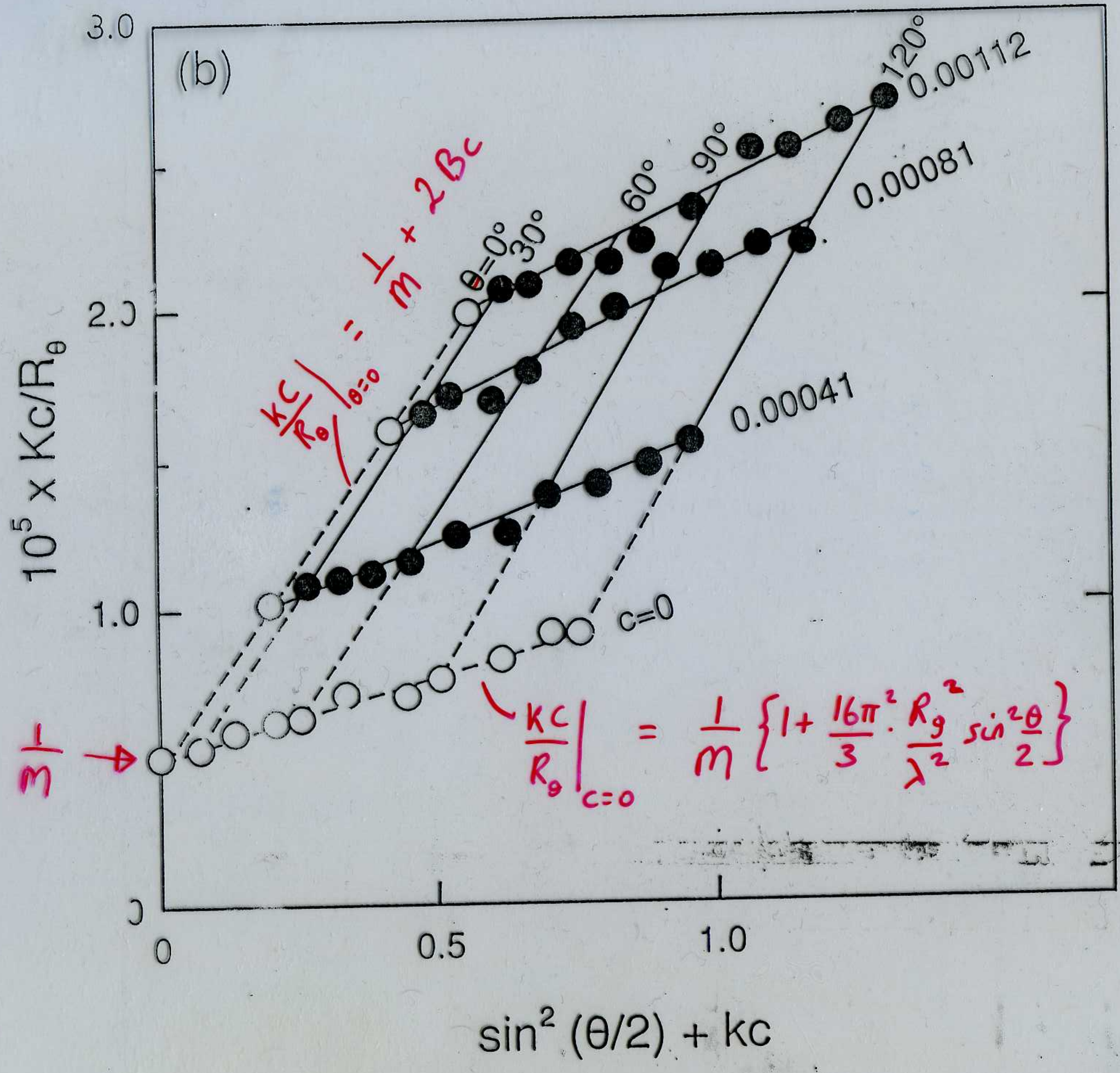
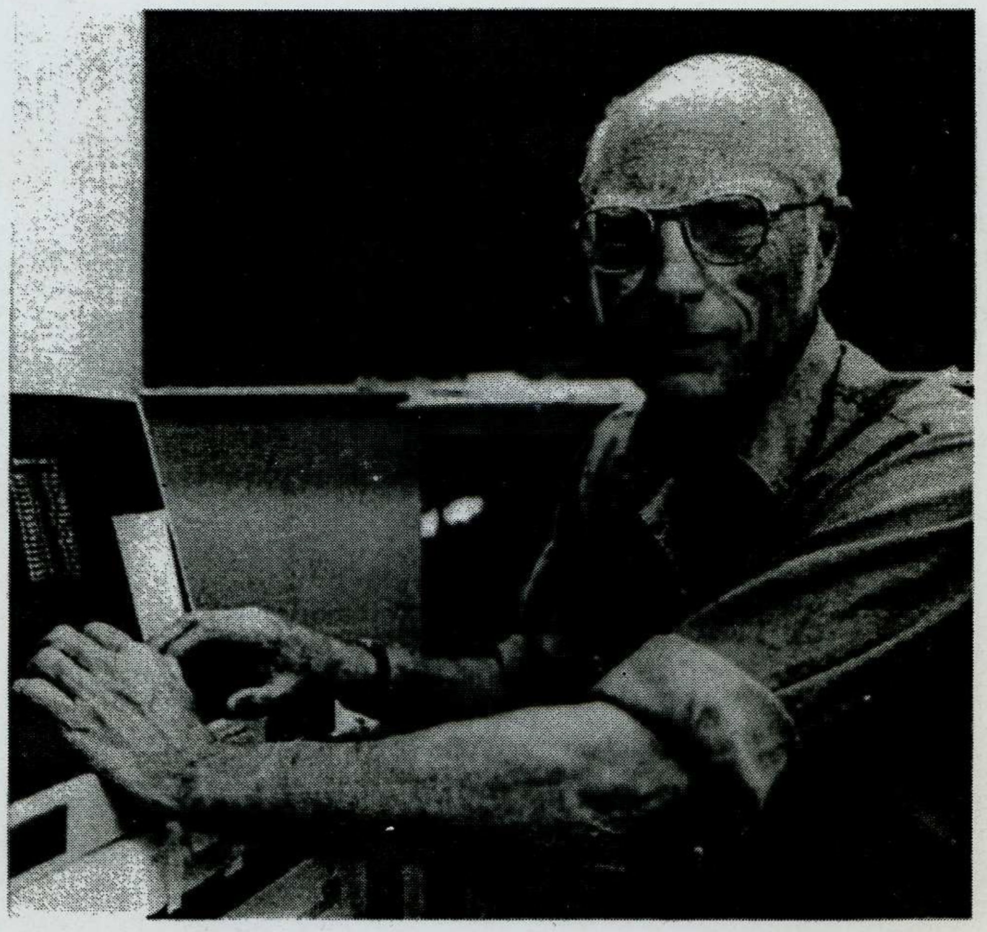


FIG 1(b)

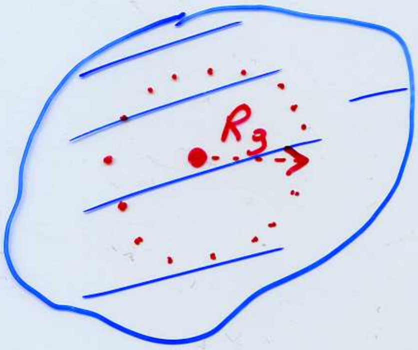


Bruno H. Zimm

CONFORMATIONAL ANALYSIS : "RADIUS OF GYRATION" R_g

Besides classical light scattering, R_g can also be obtained from solution x-ray scattering or neutron scattering. Why might these alternative techniques be more suitable for smaller biomolecules ($M \lesssim 40000$)?

RADIUS OF GYRATION R_g



- root mean square distance of mass elements in a particle from centre of mass

SPHERE (radius R) :

$$R_g = \sqrt{\frac{3}{5}}\, R$$

ROD (length L)

$$R_g = \frac{L}{\sqrt{12}}$$

ELLIPSOID (semi-axes a, b, c)

$$R_g = \sqrt{\frac{a^2+b^2+c^2}{5}}$$

(nb. prolate $c = b$
oblate $c = a$)

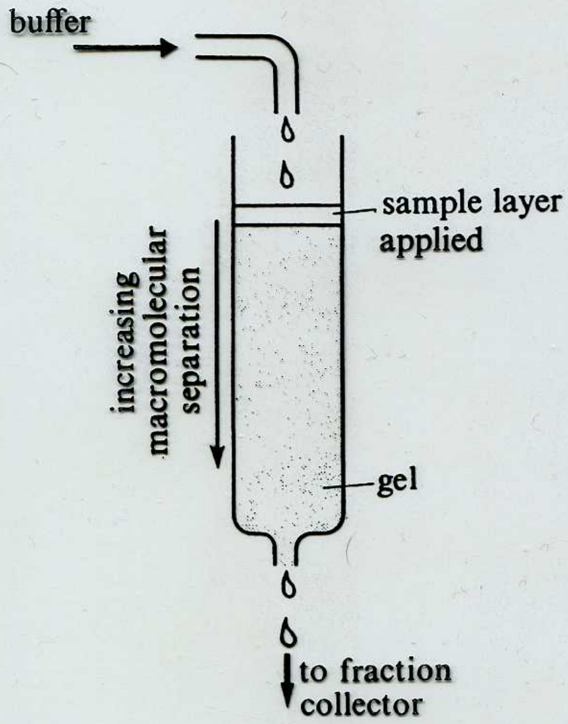
RANDOM COIL (mean square end-to end distance R^2)

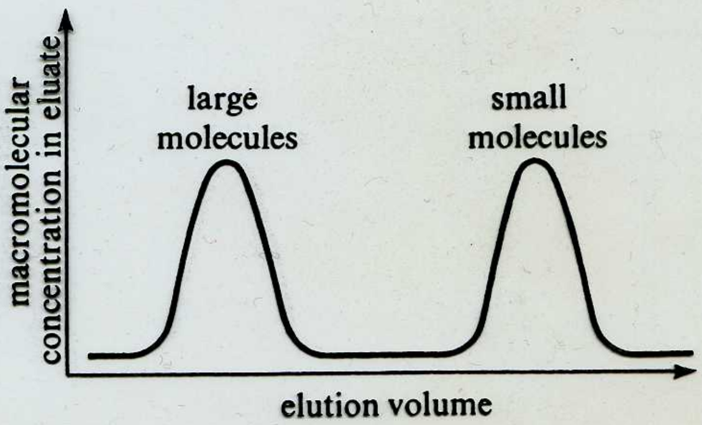
$$R_g = \langle R^2 \rangle^{\frac{1}{2}} / \sqrt{6}$$

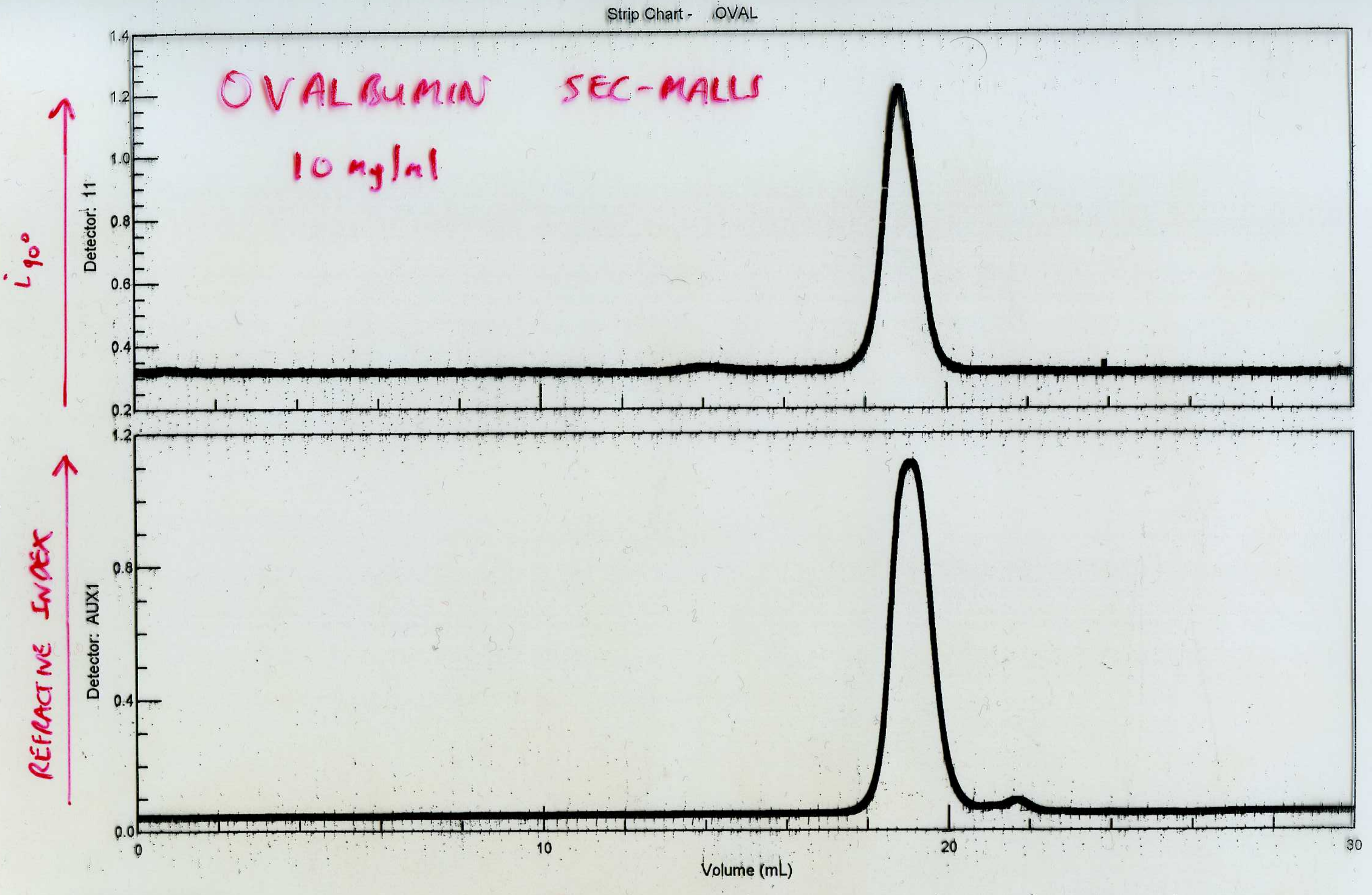
BEAD MODELS R_g = some complicated function !

Typical R_g values

Material	M	R_g (nm)	
Lysosyme	14,100	1.52	globular
Serum albumin	70,000	2.98	globular
Turnip yellow mosaic virus	5×10^6	30.0	globular
Myosin	493000	46.8	≈ rod
DNA sample	4×10^6	117.0	≈ flexible coil


buffer
sample layer applied
increasing macromolecular separation
gel
to fraction collector


macromolecular concentration in eluate
large molecules
small molecules
elution volume



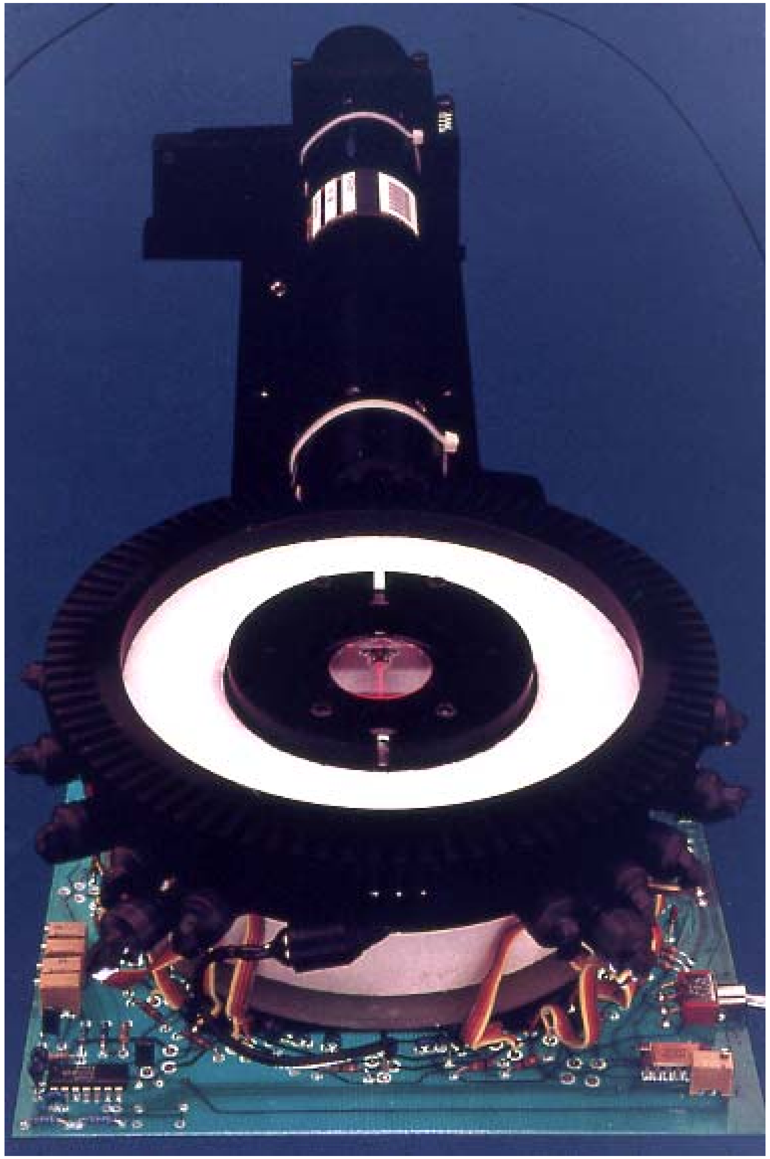
ovalbumin 10mg/ml
C:\MARCIN\10OVAL.ADF
ASTRA for Windows 4.73.04

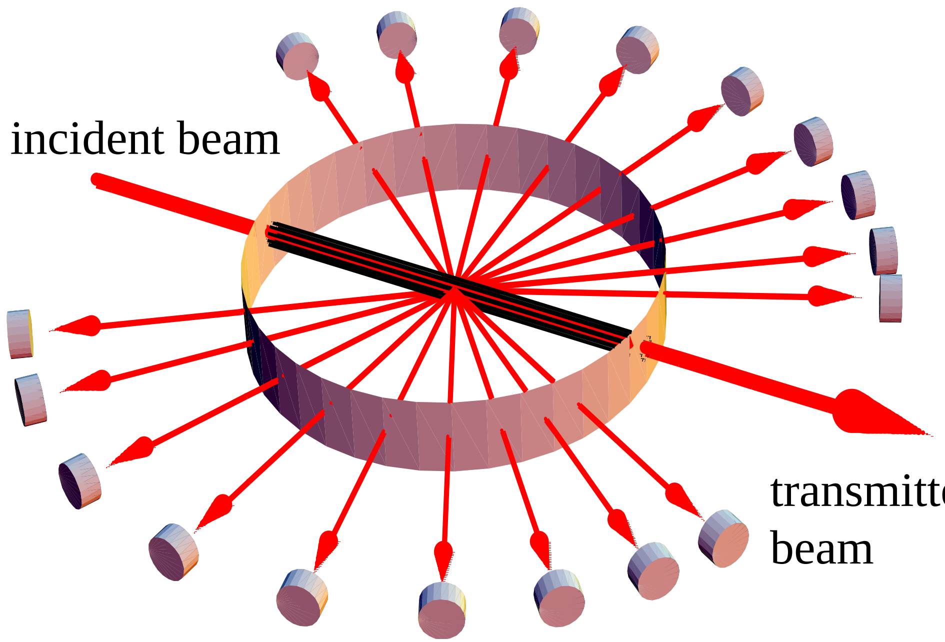
Tue Nov 25, 2003 01:43 PM

Molecular Weight: Light scattering

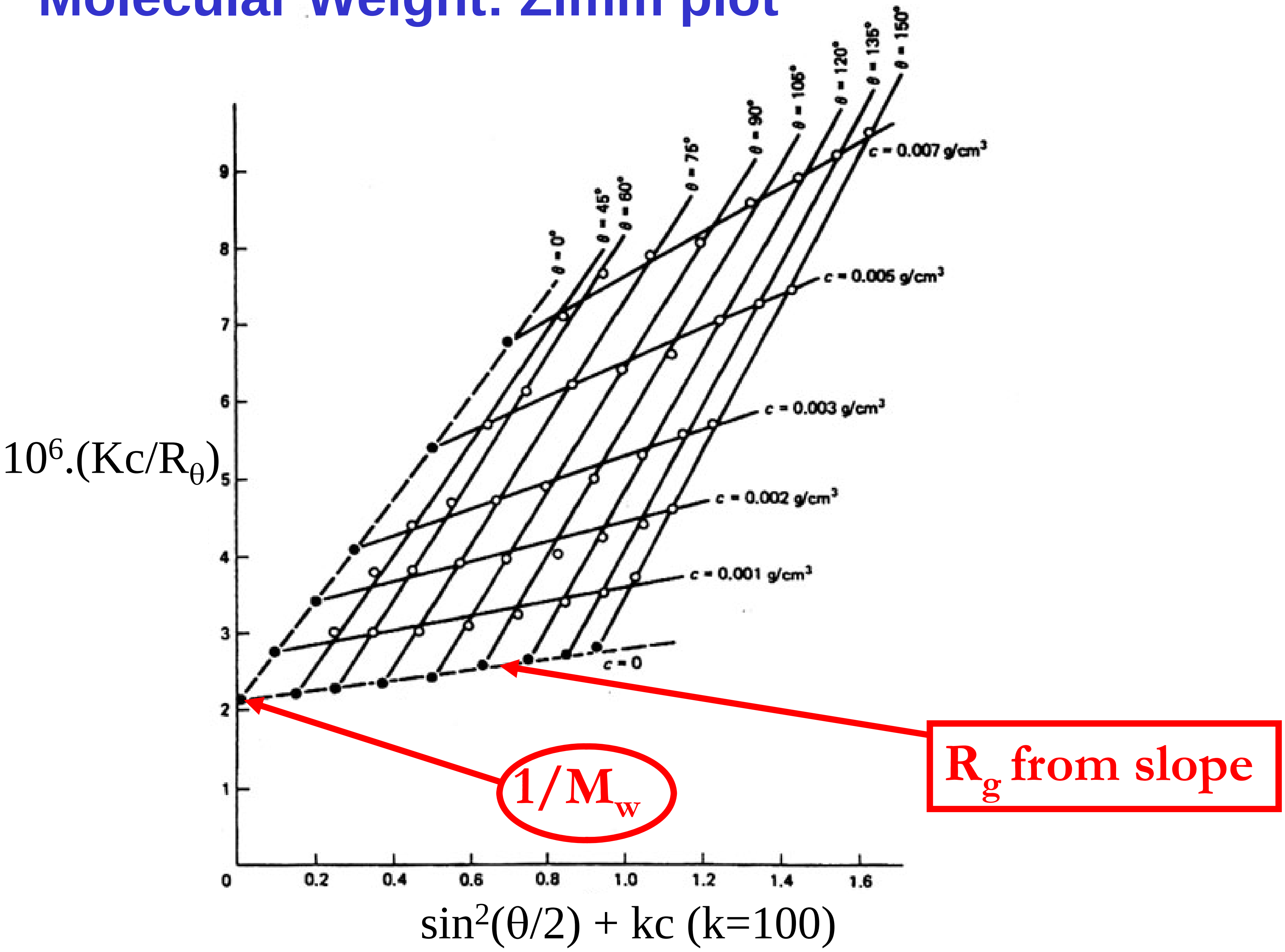
"MALLs" detector
with 120mW laser operating at 658 nm

Molecular Weight: Light scattering

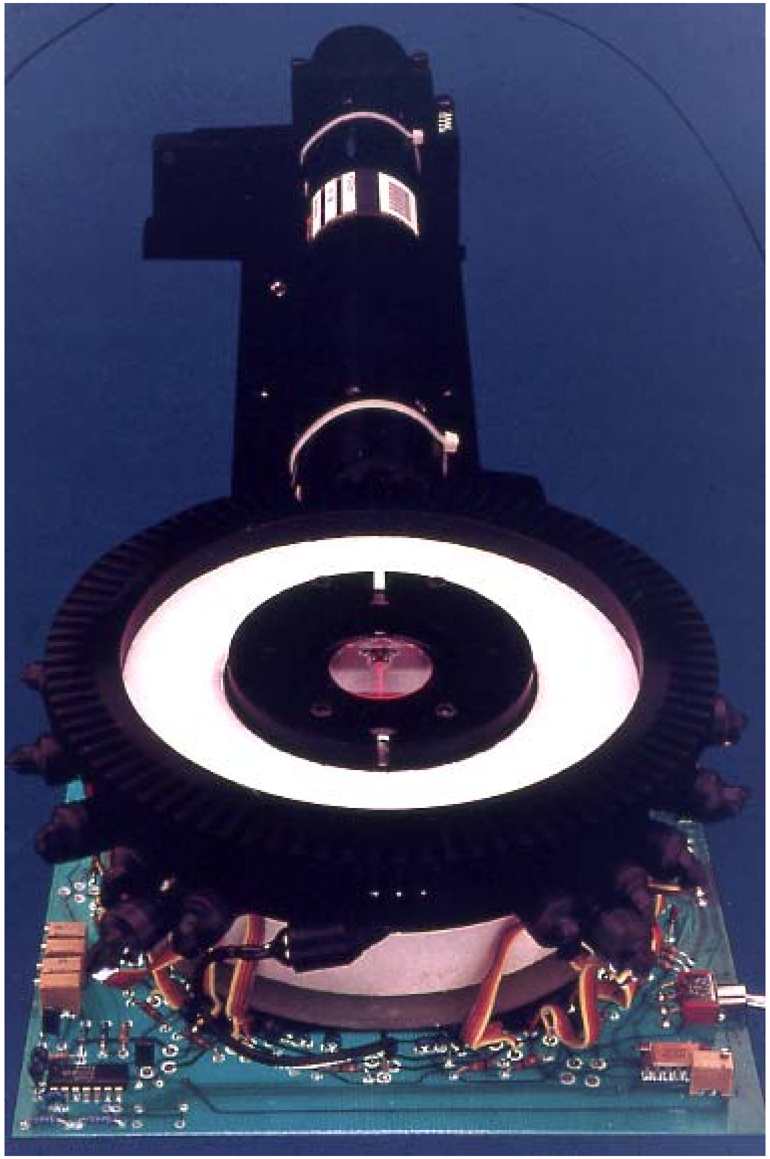


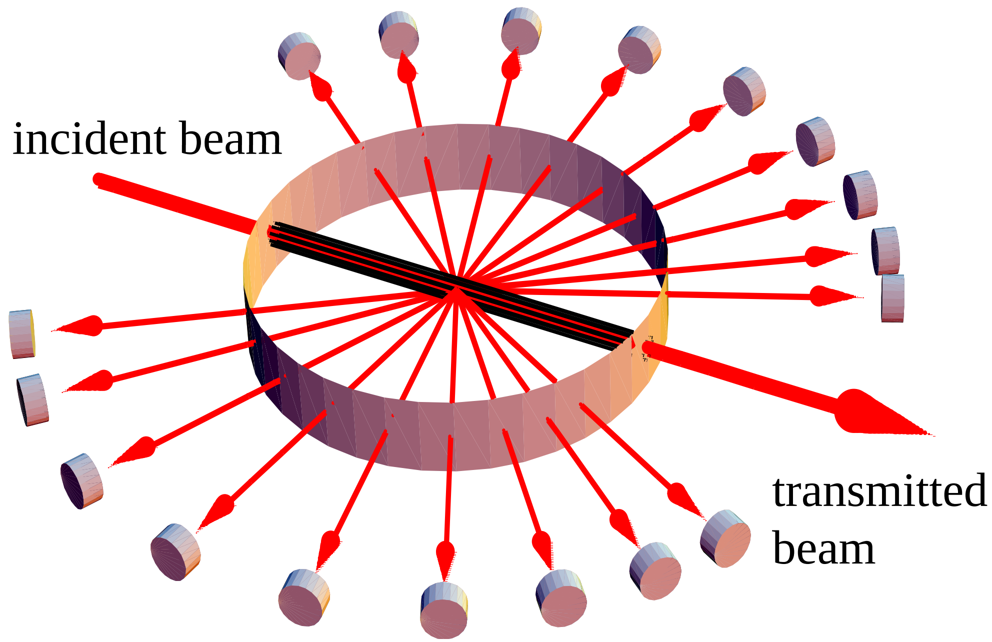


Molecular Weight: Zimm plot

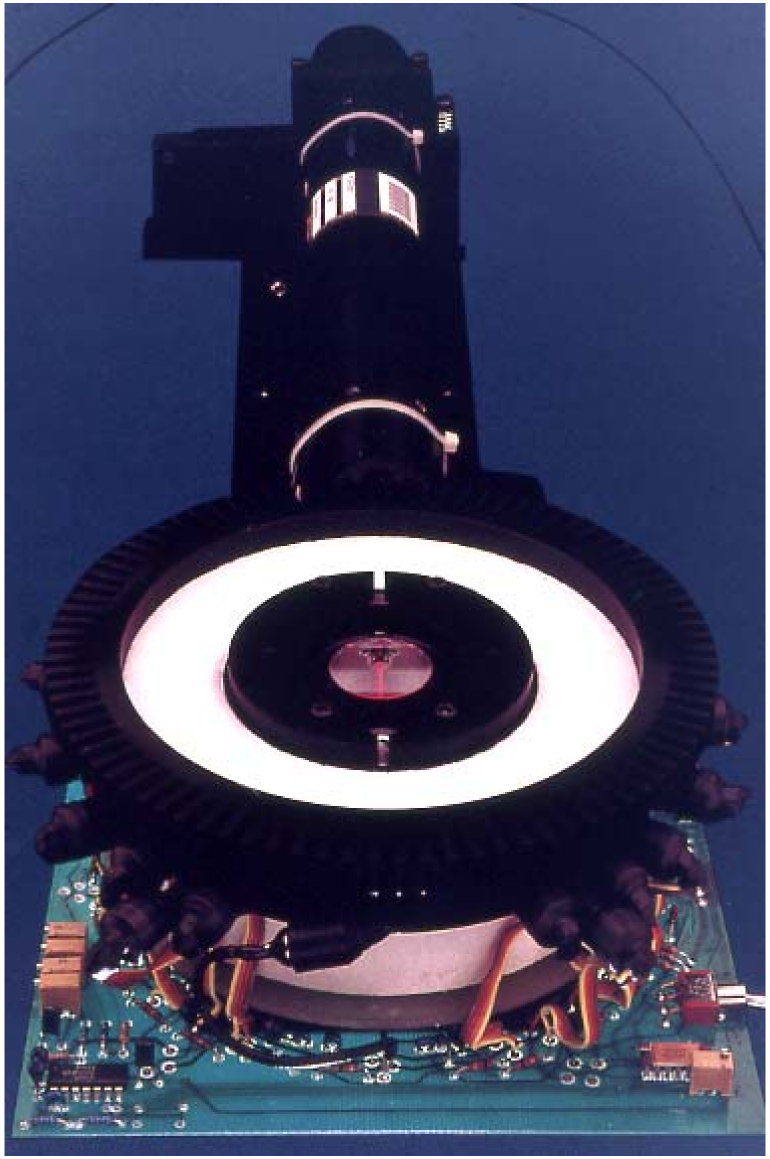


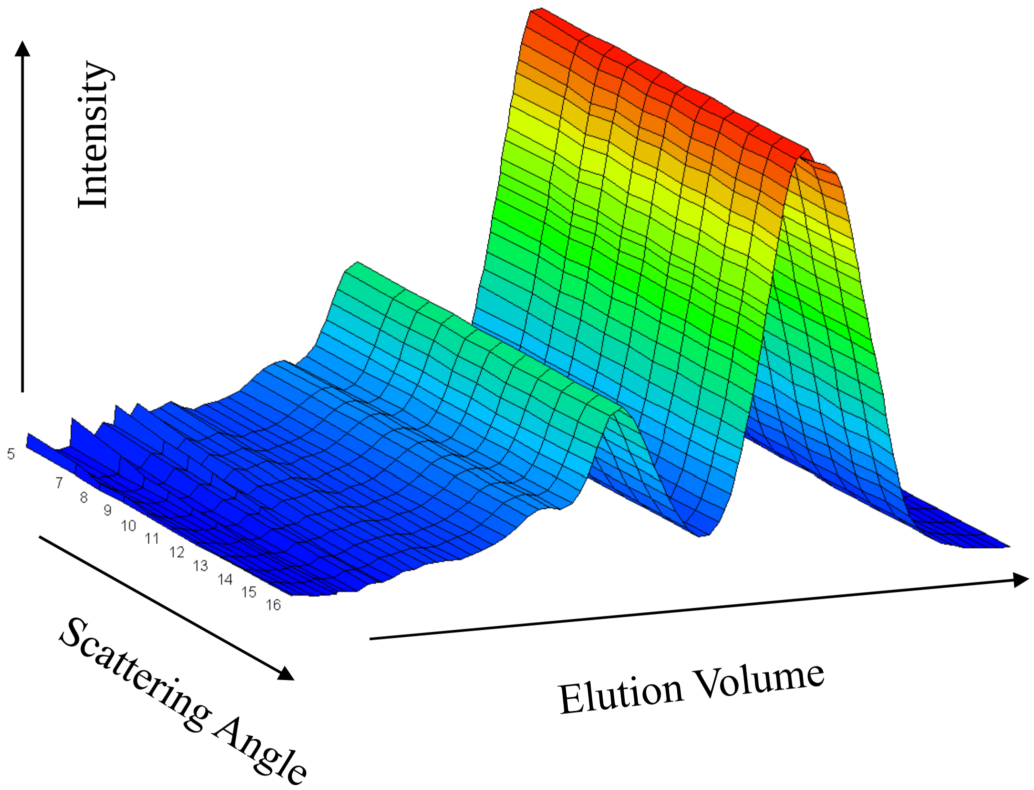
Molecular Weight: Light scattering





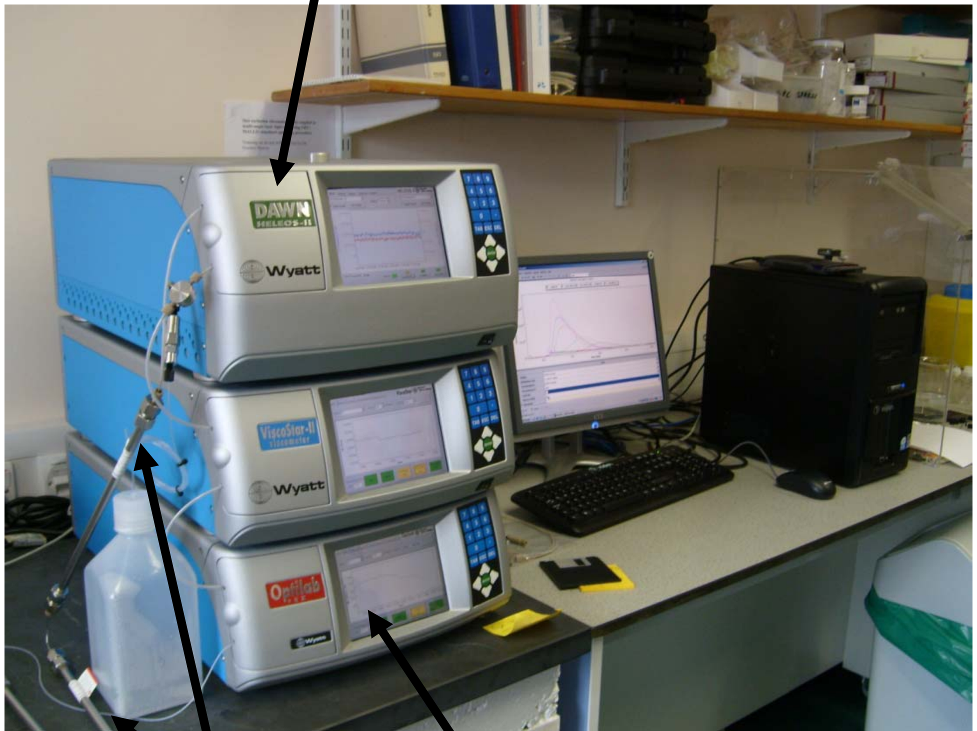
Molecular Weight: Light scattering





Molecular Weight: SEC–MALLS

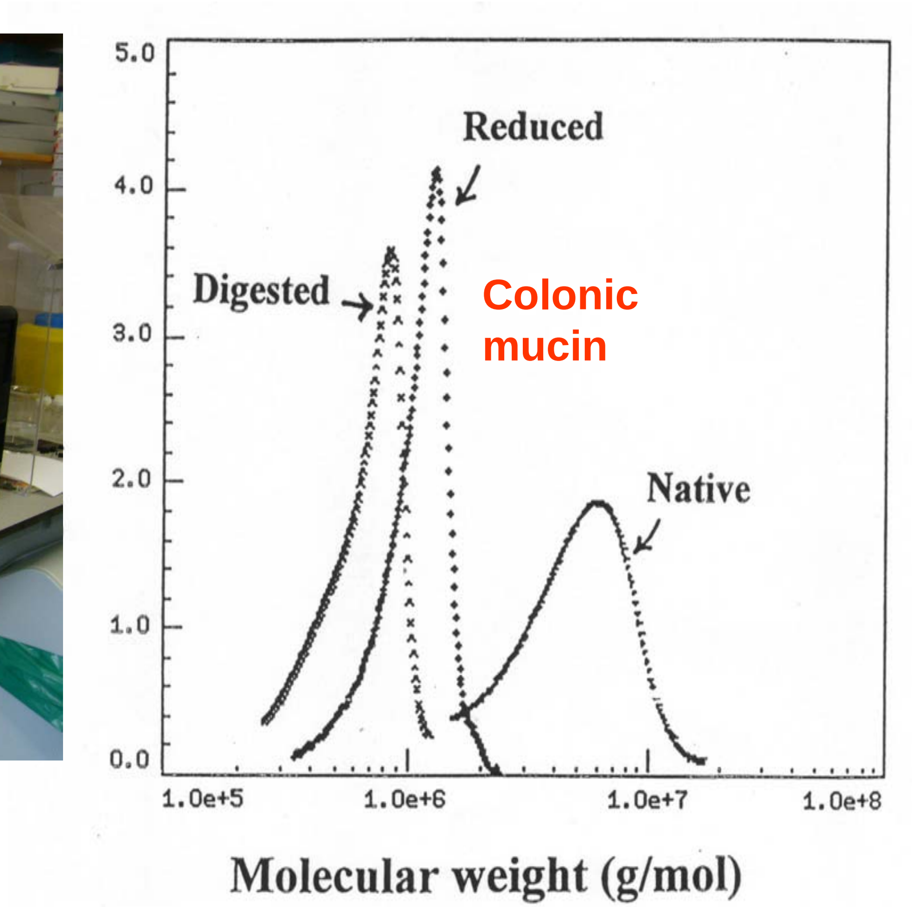
MALLs detector



SEC - columns

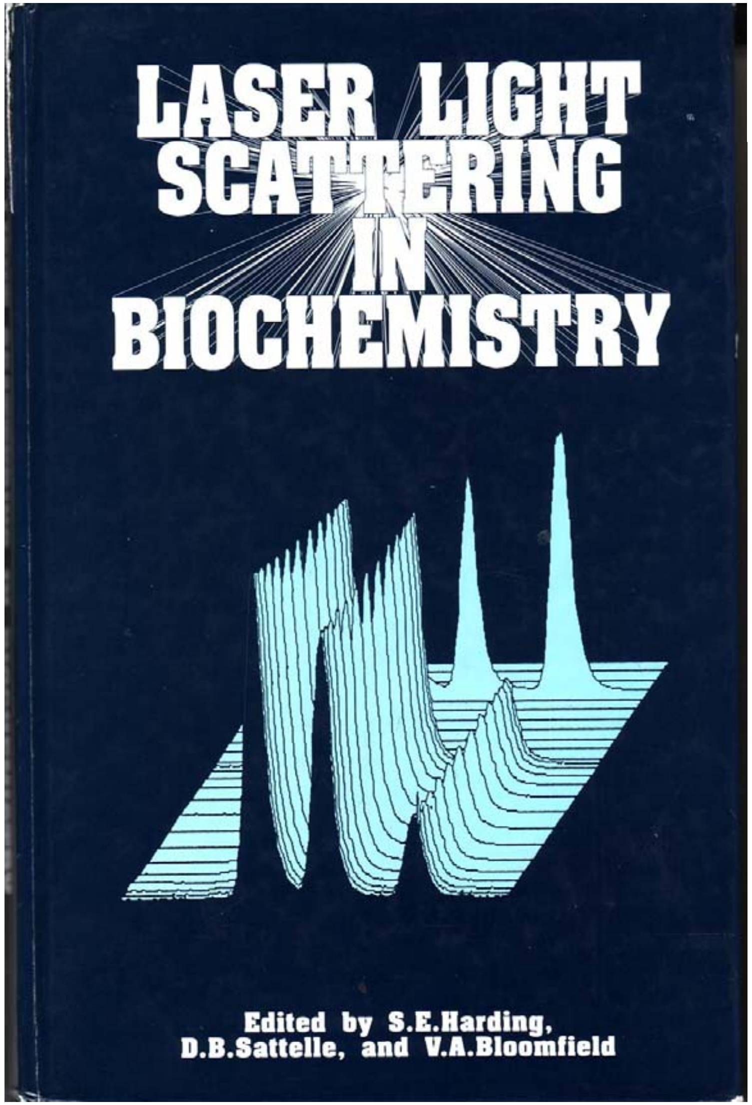
Concentration detector

Molecular Weight: SEC–MALLS



Fogg FJJ et al, *Biochemical Journal.*1996

Molecular Weight: SEC–MALLS



3

Combined Differential Light Scattering with Various Liquid Chromatography Separation Techniques

By Philip J. Wyatt

WYATT TECHNOLOGY CORPORATION, SANTA BARBARA, CALIFORNIA 93130-3003, U.S.A.

1. INTRODUCTION

The combination of light scattering measurements with various particle/molecular separation techniques often permits an unparalleled characterization of the separated particles. In a sense, this is but an application of the so-called "inverse scattering" problem[1,2,3], *i.e.* from measurements of the light scattering

Biochemical Society Transactions 19 (1991) 510-511

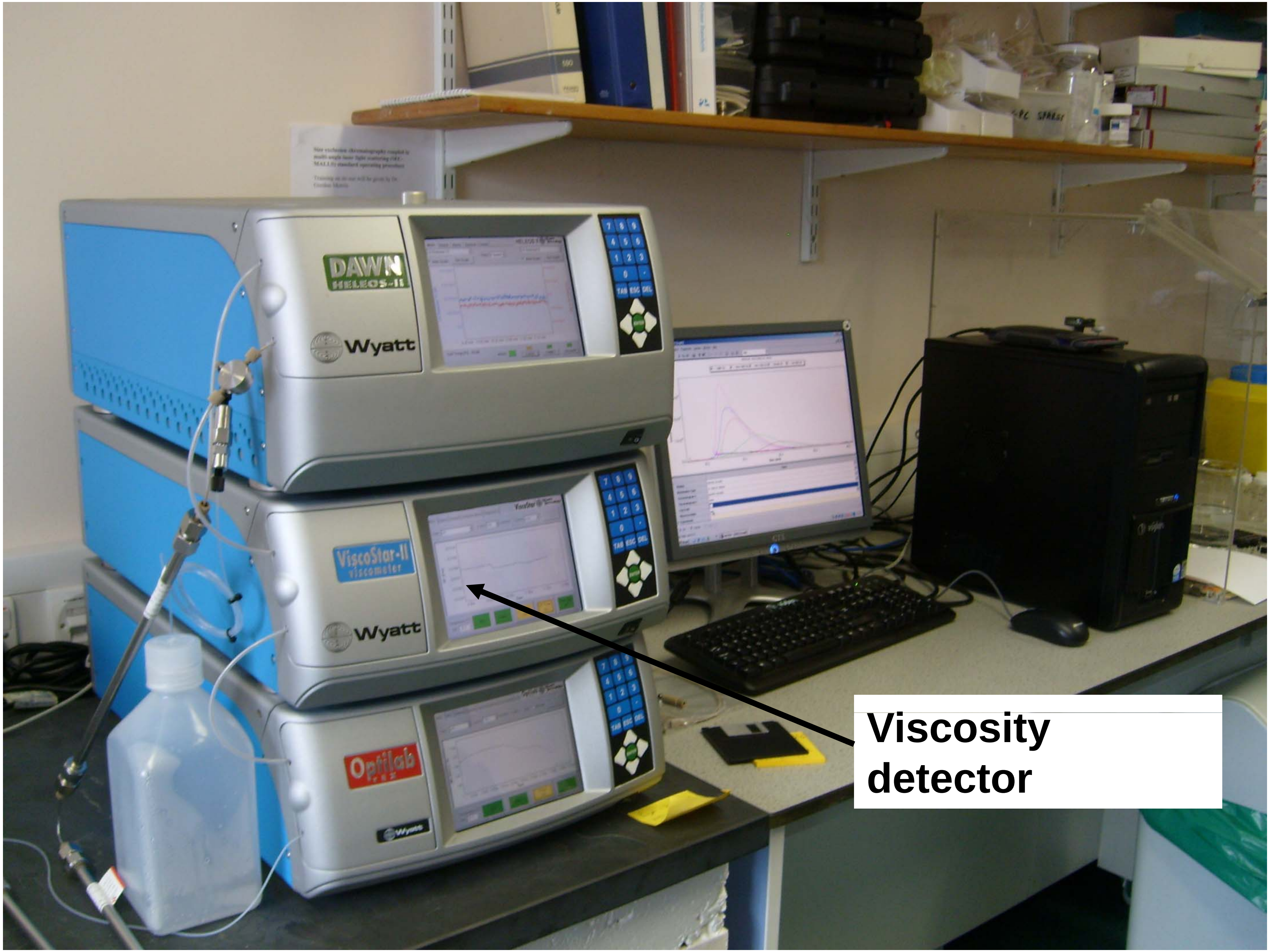
Gel permeation chromatography–multi-angle laser light scattering characterization of the molecular mass distribution of 'Pronova' sodium alginate

J. C. Horton, S. E. Harding and J. R. Mitchell

University of Nottingham, Department of Applied Biochemistry and Food Science, School of Agriculture, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, U.K.

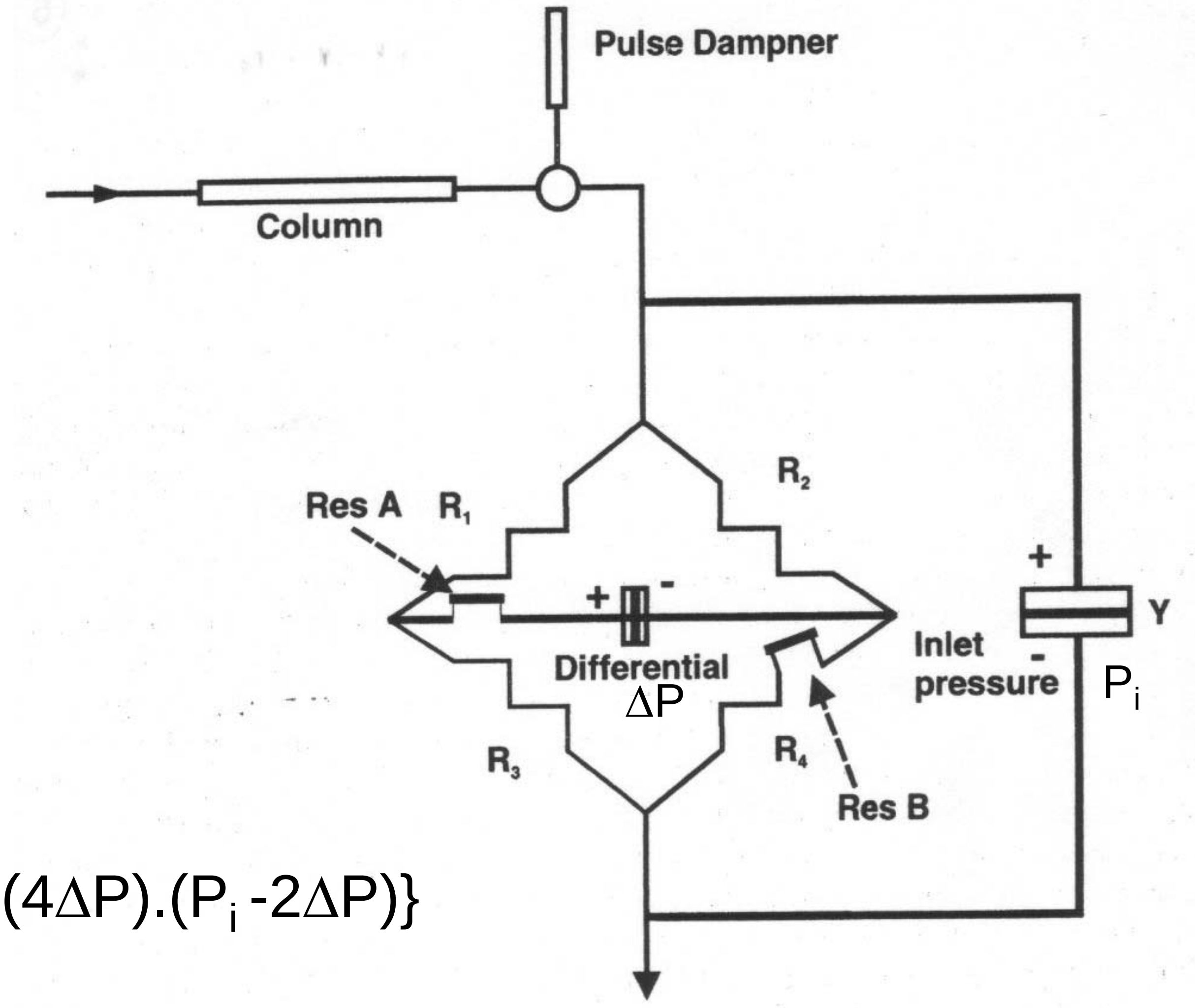
A relatively recent innovation in total intensity laser light scattering has been to replace the isolated

fers of ionic strengths (I) of 0.1 M and 0.3 M with concentrations (c) in the range 0.5–5.0 mg/ml.



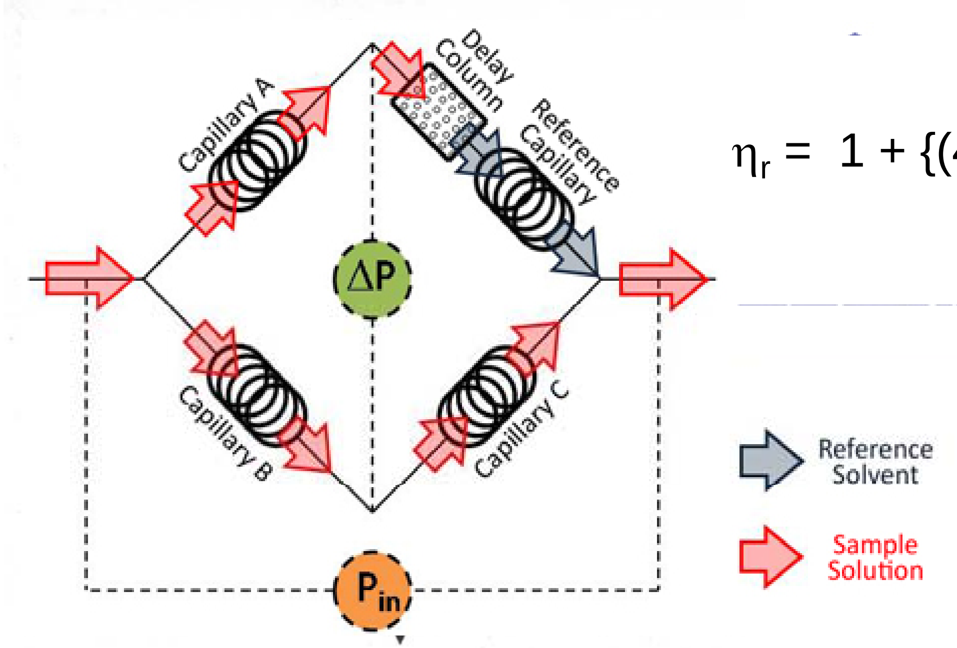
DAWN
HELEOS-II
Wyatt
ViscoStar-II
viscometer
Wyatt
Optilab
Wyatt
Viscosity detector

Pressure Imbalance Viscometer:



$$\eta_r = 1 + \{(4\Delta P).(P_i - 2\Delta P)\}$$

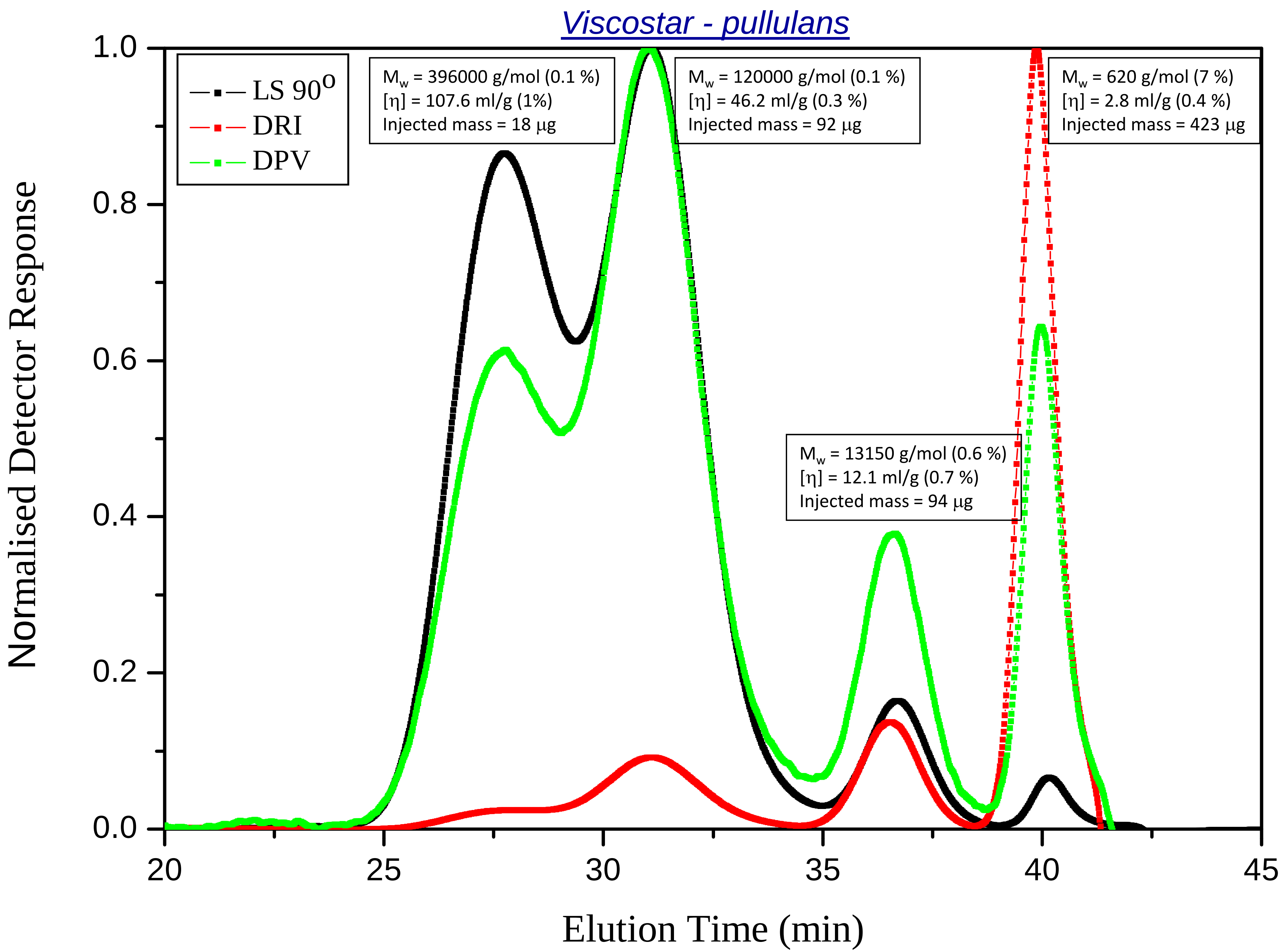
Pressure Imbalance Viscometer:



$$\eta_r = 1 + \{(4\Delta P).(P_i - 2\Delta P)\}$$

Wyatt Technology Viscostar System

Viscotek System

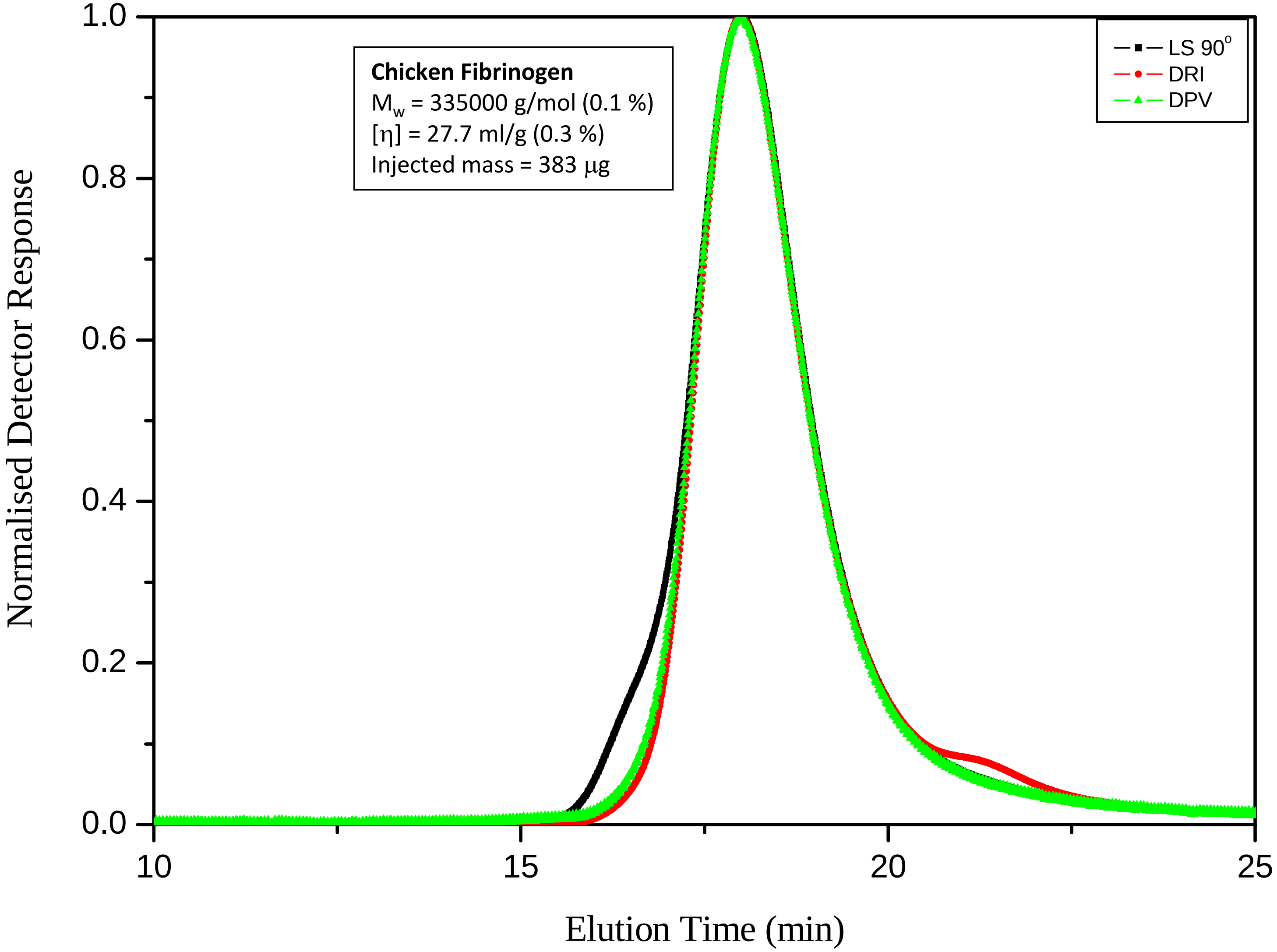


Viscostar - pullulans
LS 90°
DRI
DPV
M_w = 396000 g/mol (0.1 %)
[η] = 107.6 ml/g (1%)
Injected mass = 18 μg
M_w = 120000 g/mol (0.1 %)
[η] = 46.2 ml/g (0.3 %)
Injected mass = 92 μg
M_w = 620 g/mol (7 %)
[η] = 2.8 ml/g (0.4 %)
Injected mass = 423 μg
M_w = 13150 g/mol (0.6 %)
[η] = 12.1 ml/g (0.7 %)
Injected mass = 94 μg
Normalised Detector Response
Elution Time (min)
0.0
0.2
0.4
0.6
0.8
1.0
20
25
30
35
40
45

….Viscostar - Fibrinogen

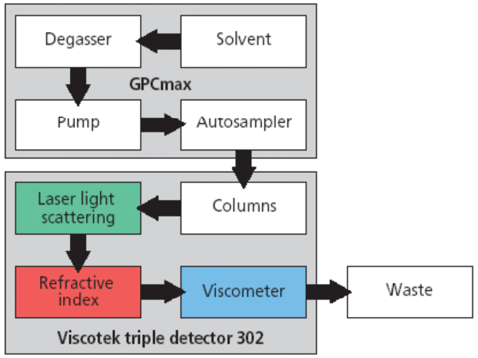
Hydrodynamic and mass spectrometry analysis of nearly-intact human fibrinogen, chicken fibrinogen, and of a substantially monodisperse human fibrinogen fragment X

Barbara Cardinali[a], Aldo Profumo[a], Anna Aprile[a], Olwyn Byron[b], Gordon Morris[c], Stephen E. Harding[c], Walter F. Stafford[d], Mattia Rocco[a,*]



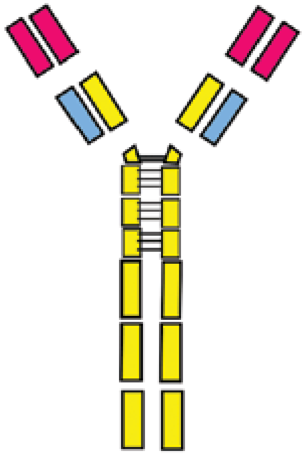
Identifying Differences in Solution Conformations of Two Chimeric IgG3 Antibodies through Triple Detection SEC

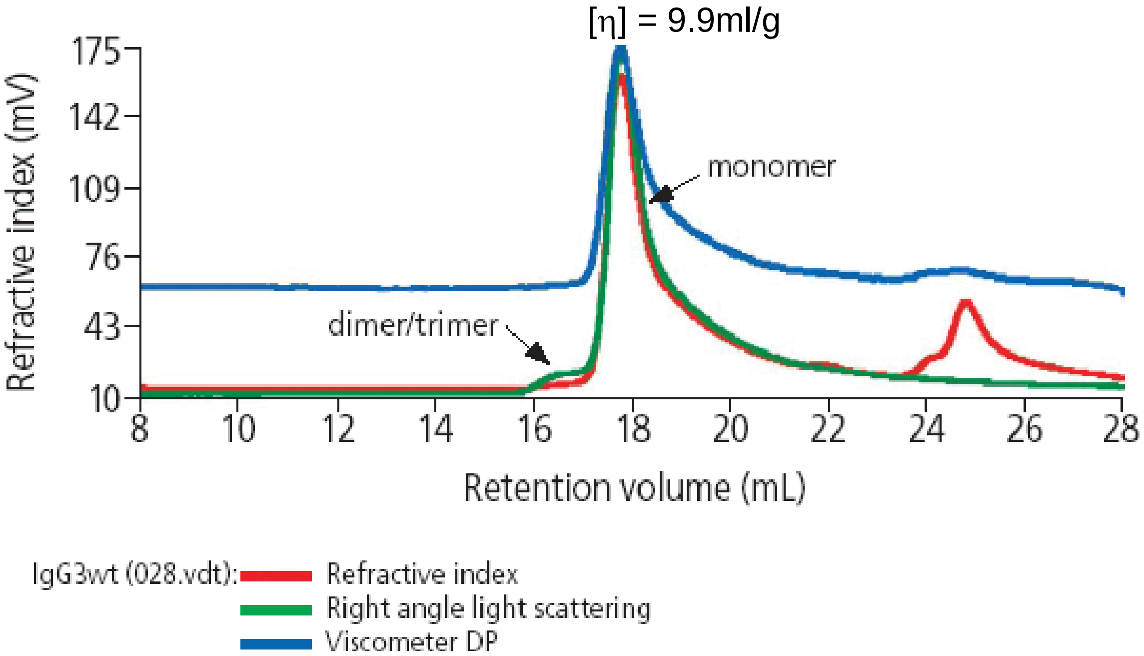
Jan 1, 2006
By: Emma Longman, Stephen E. Harding, Nicola Marheineke
LCGC NORTH AMERICA
Volume 24, Issue 1



Viscotek

IgG3wt





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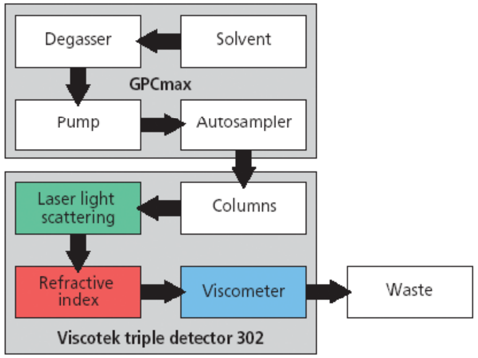
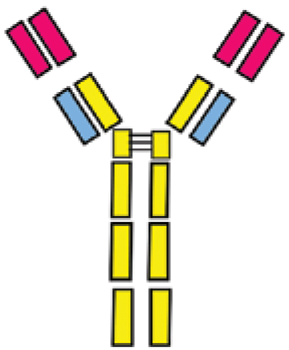


IgG3m15



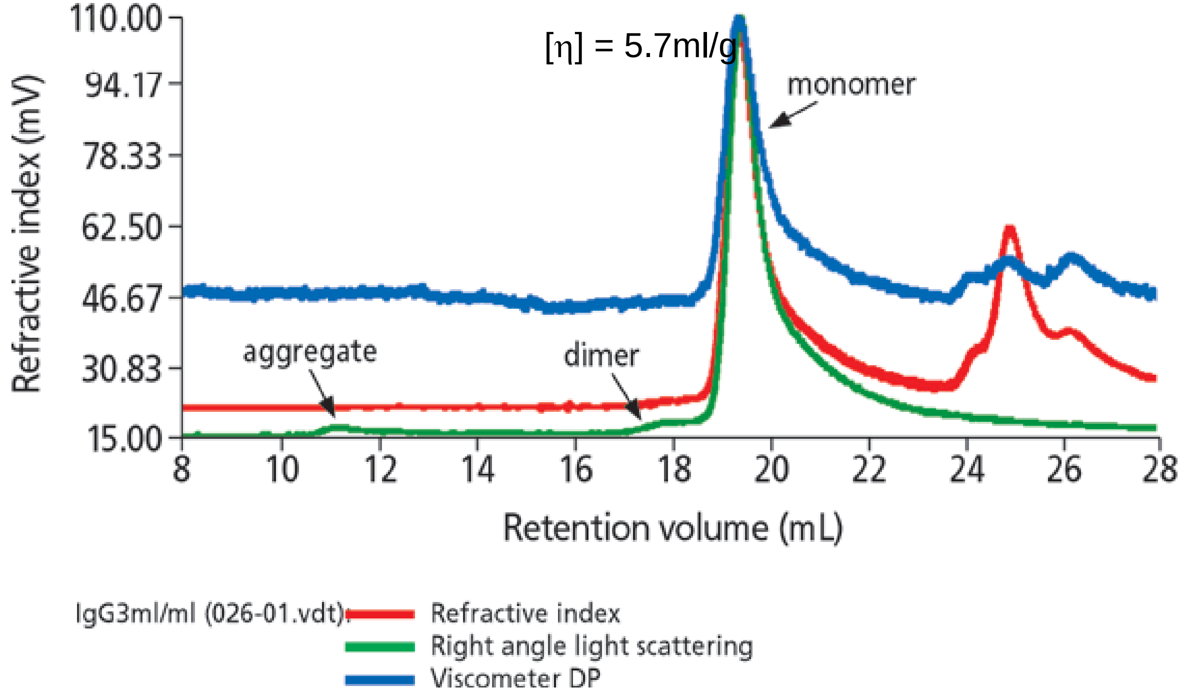
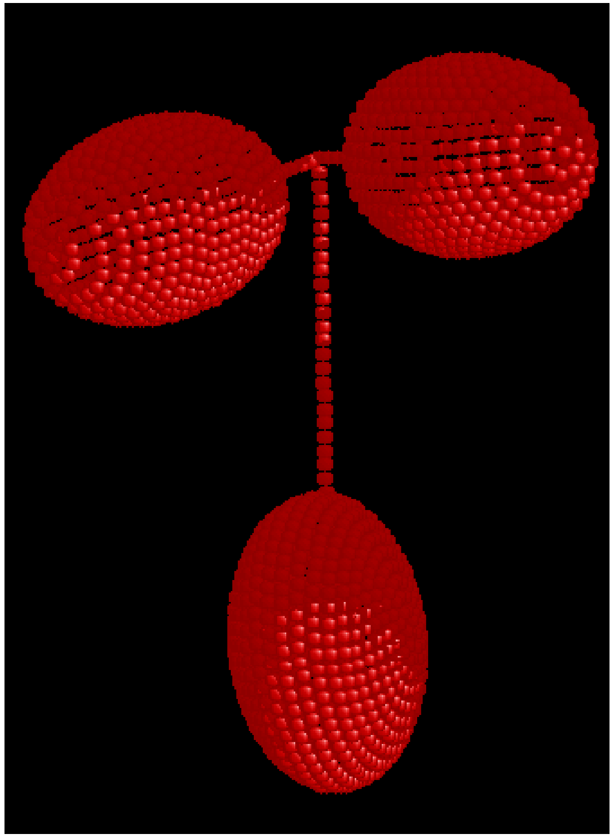
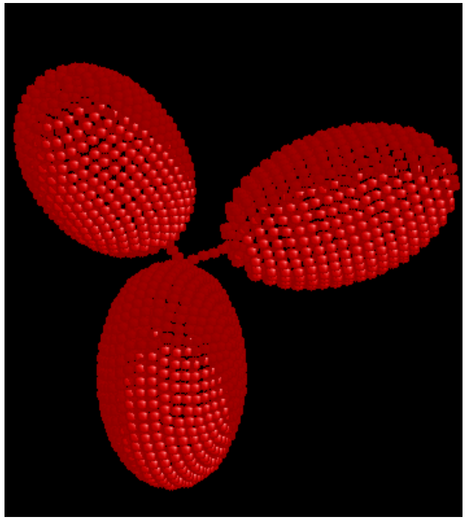


Table I: Results determined through triple detection of IgG3wt and IgG3m15 antibodies.

Antibody	Molecular weight from literature (Da)	Experimental molecular weight (da)	Intrinsic viscosity (ML/g)	Viscosity Increment	Hydrodynamic radius (nm)
IgG3wt	160 000	170 300	9.9	7.5	6.4
IgG3m15	150 000	149 700	5.7	4.3	5.1



A bead –shell model of the of IgG3 wild type



A bead-shell model of a mutant IgG3

Lu et al, Biophysical Journal, 2006

Follow up bibliography:

1. On-line tutorials from: Wyatt Technology and Viscotek corporation (see their web sites)
2. Harding, S.E., Sattelle, D.B. & Bloomfield, VA. Eds (1992) *Laser Light Scattering in Biochemistry* Royal Soc. Chem. Cambridge – Particularly chapters by Wyatt and Rollings