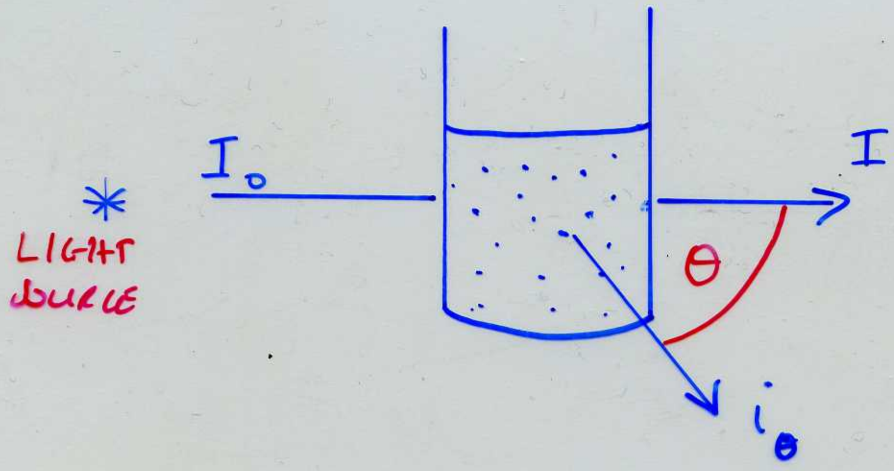
LIGHT SCATTERING



W19

2 MAIN TYPES

① CLASSICAL or "STATIC" LIGHT SCATTERING

- Measure scattered intensity i_θ as a function of angle θ ⟶ 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 ⟶ D (diffusion coefficient)

CLASSICAL LIGHT SCATTERING

.... for characterisation of biomolecular <u>molecular weights</u> and <u>conformations</u> (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

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 18[illegible], aged 28, photographed by himself w[illegible] [illegible] wet collodion plate

1881 : RAYLEIGH - SINGLE PARTICLE THEORY {SMALL SCATTERERS}

1890 :	LORENZ	}	GENERAL THEORY
1908 :	MIE		
:	DEBYE		

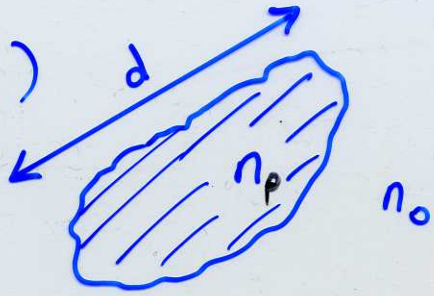
1914 :	RAYLEIGH	}	APPROXIMATE THEORY
1915 :	DEBYE		
1925 :	GANS		

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 $\quad d \lesssim \dfrac{\lambda}{20}$ $\quad$ ($M \lesssim 40000$)
- 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 $\quad d \gtrsim \lambda$ $\quad$ 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 : $\frac{2\pi^2 n_0^2 (dn/dC)^2}{N_A \lambda^4}$

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

R_G: "radius of gyration".

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

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

ZIMM PLOT : ALGINATE POLYSACCHARIDE ($M \sim 200{,}000$)

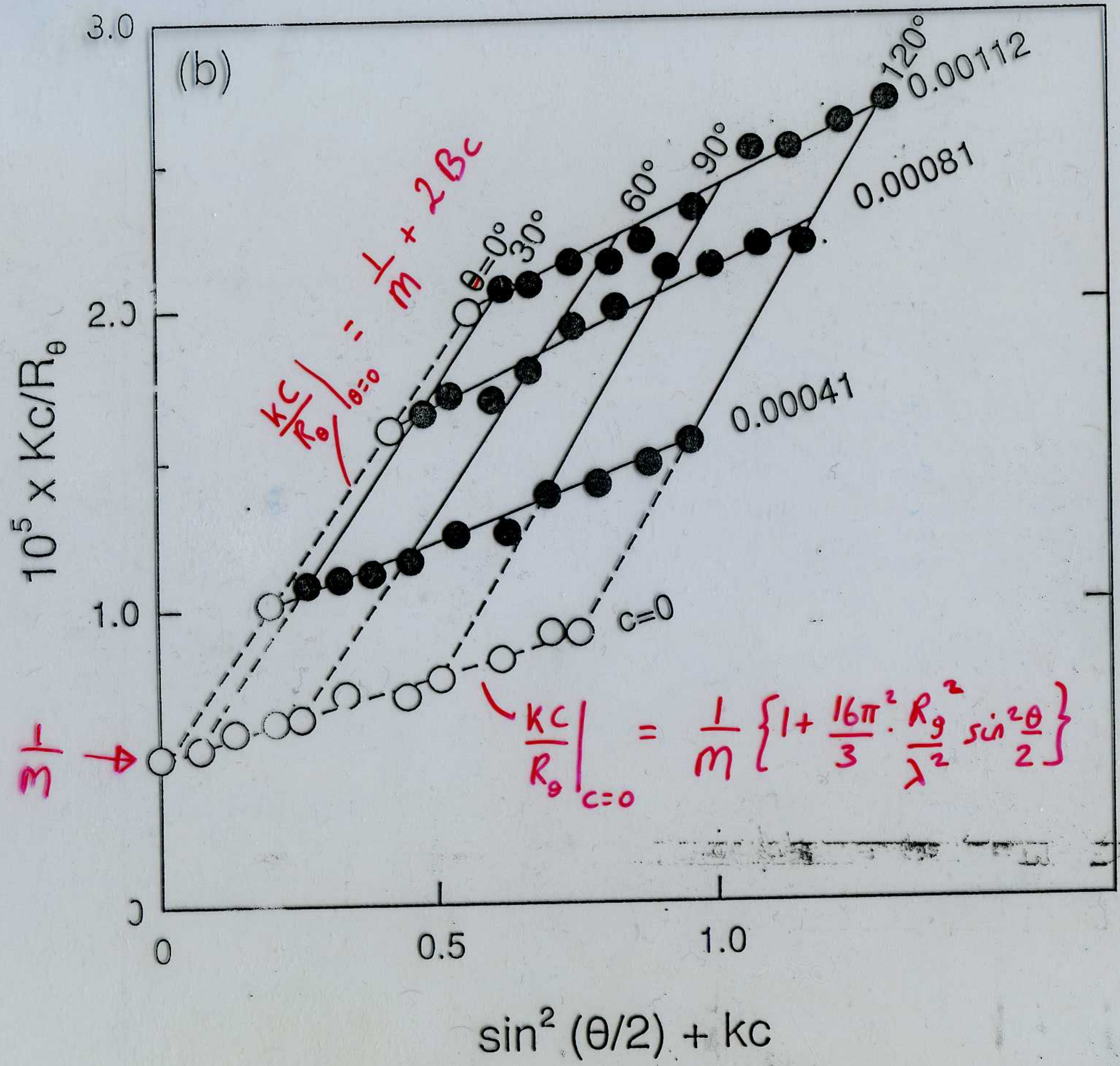
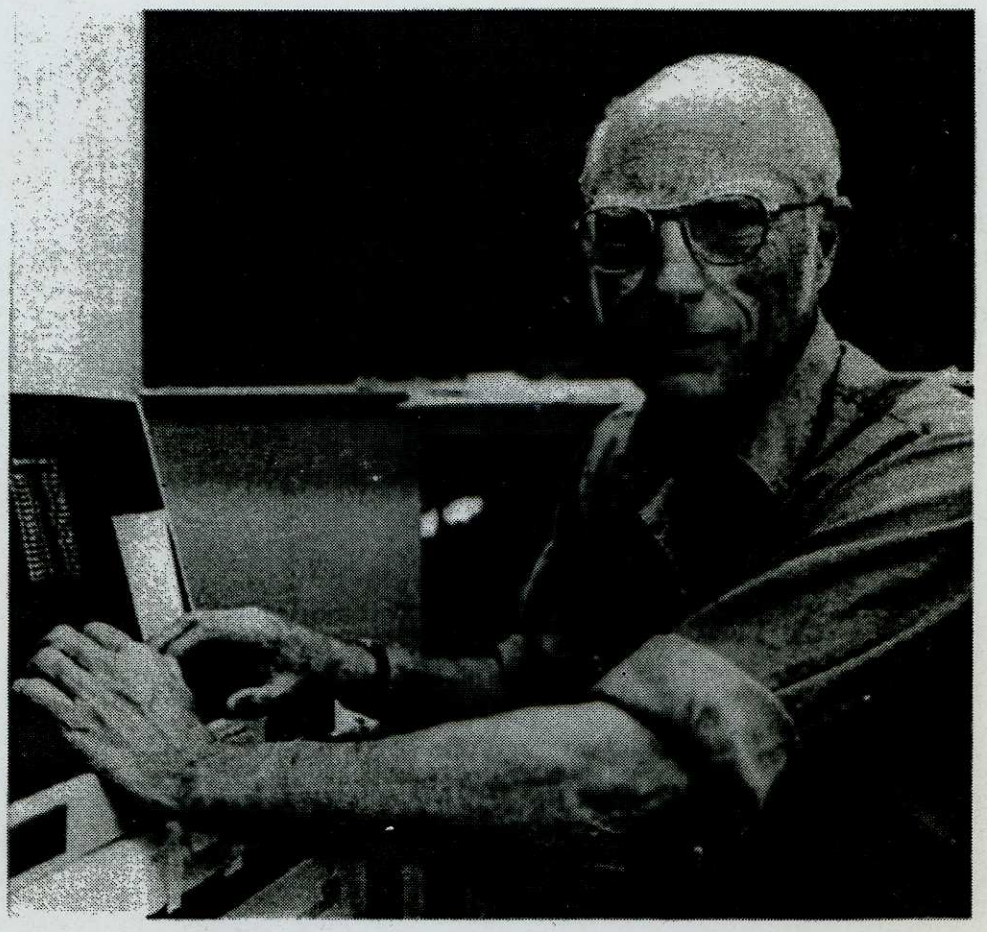


FIG 1(b)

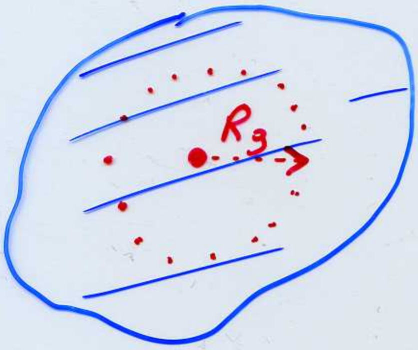


Bruno H. Zimm

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

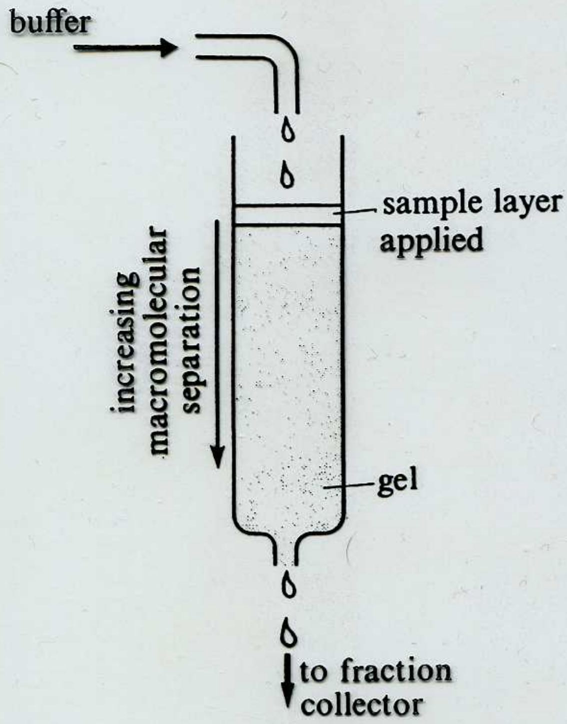
(nb. prolate c = b
oblate c = a)

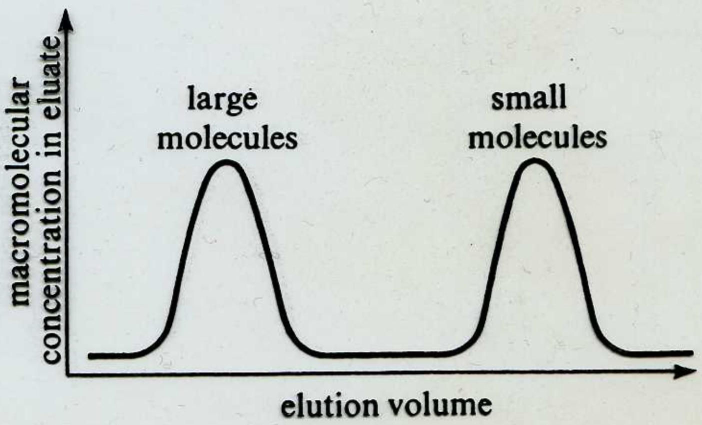
RANDOM COIL (mean square end-to end distance R^2) $R_g = \langle R^2 \rangle^{\frac{1}{2}} / \sqrt{6}$

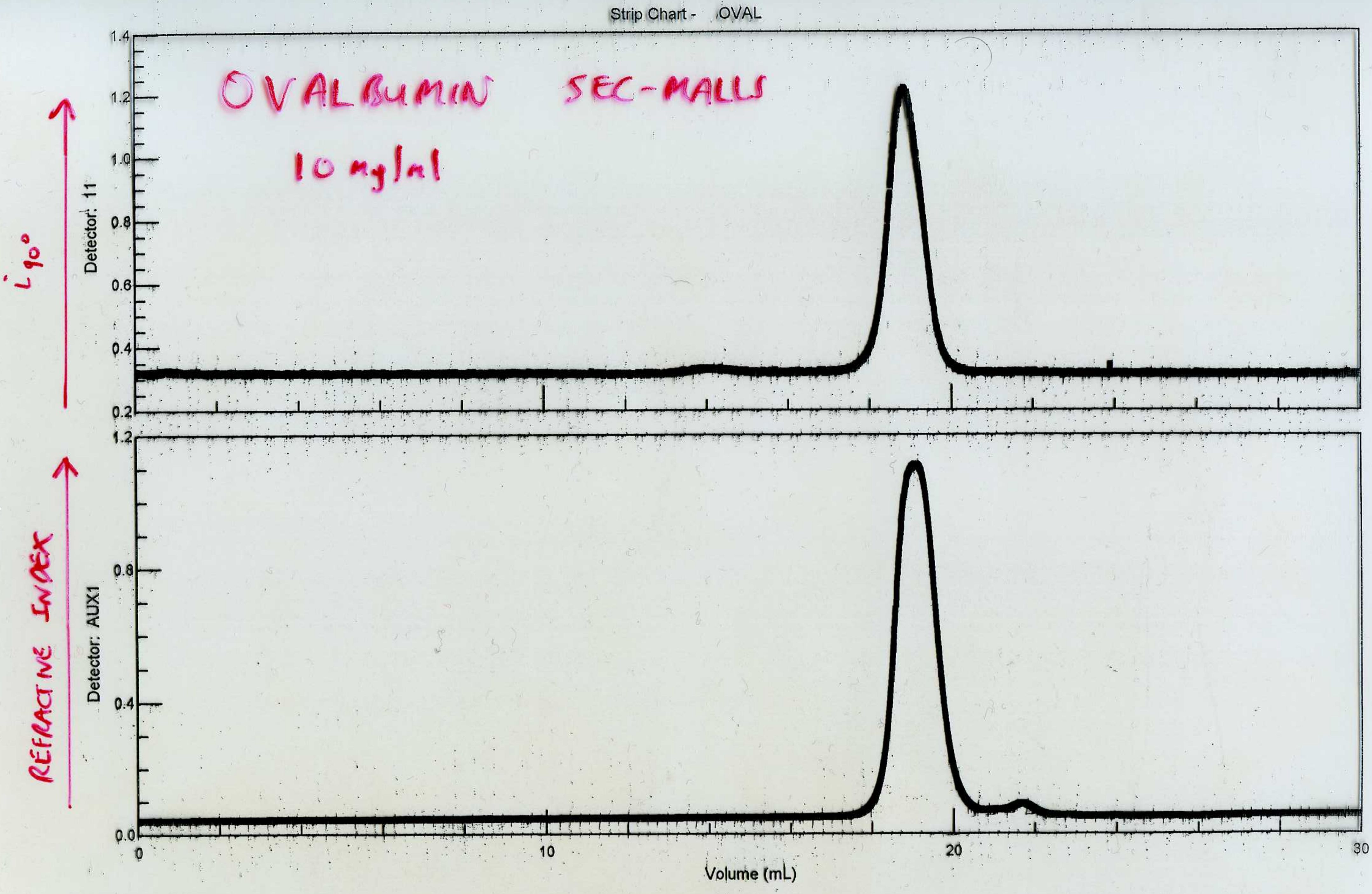
BEAD MODELS R_g = some complicated function !

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

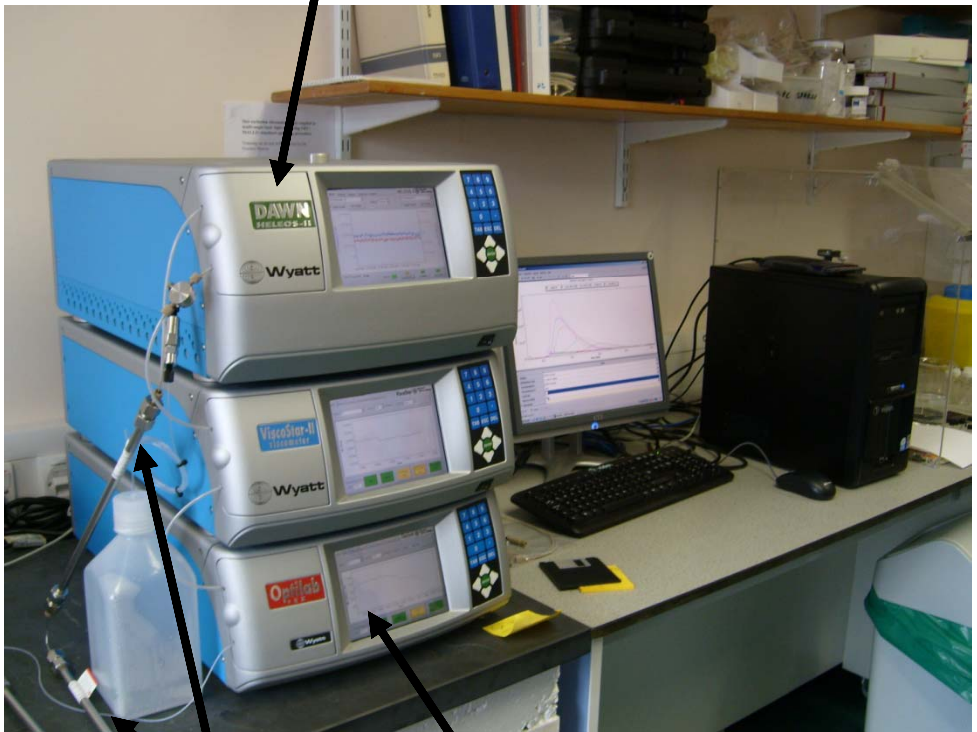


ovalbumin 10mg/ml
C:\MARCIN\10OVAL.ADF
ASTRA for Windows 4.73.04

Tue Nov 25, 2003 01:43 PM

Molecular Weight: SEC–MALLS

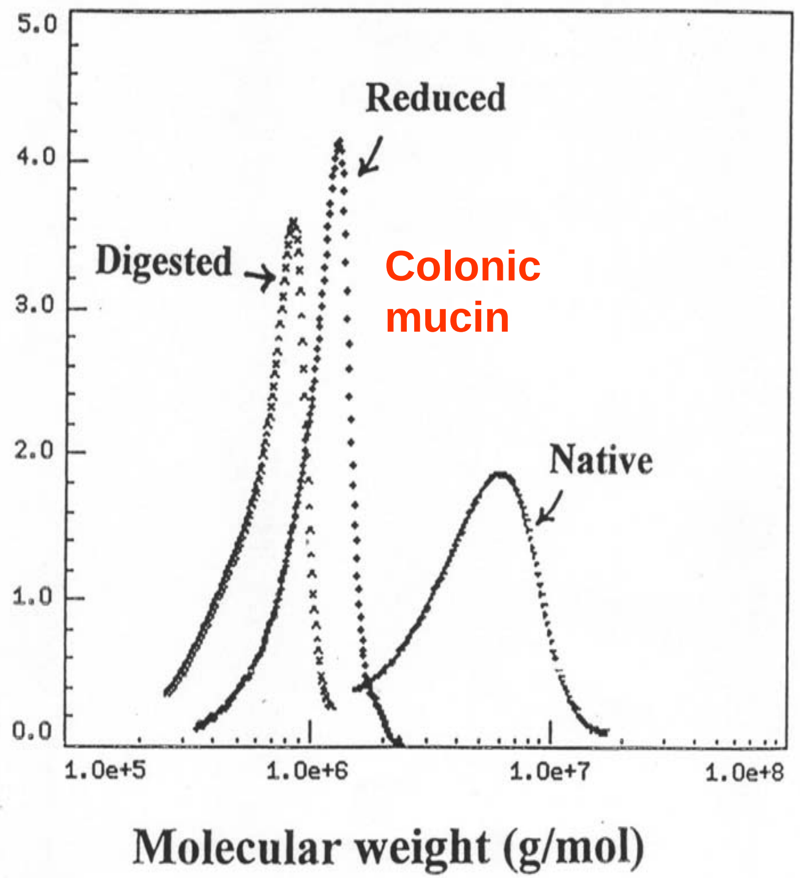
MALLs detector



SEC - columns

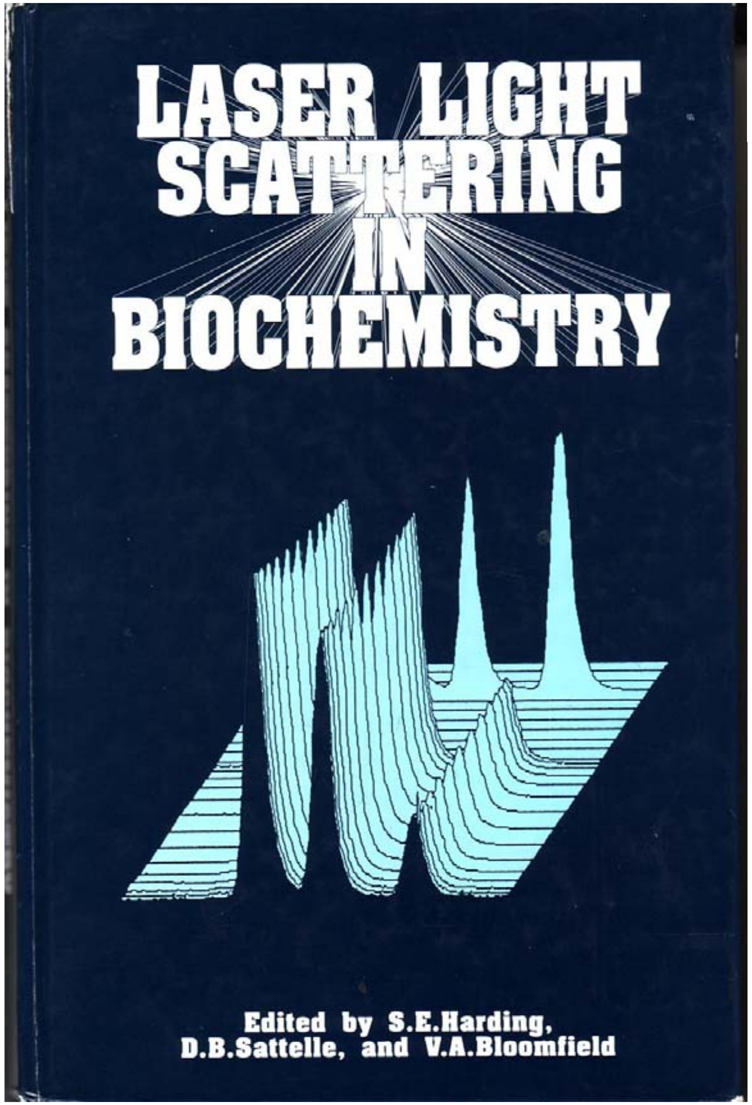
Concentration detector

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

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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.

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