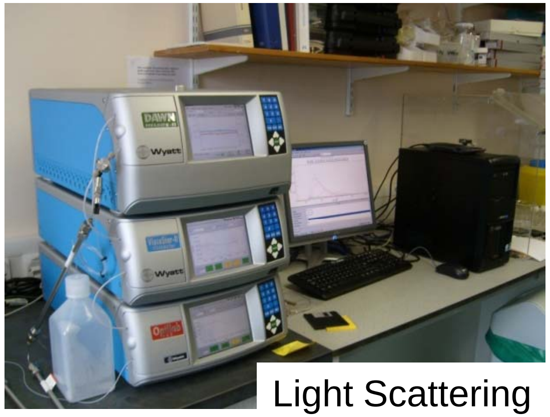
Sizes, shapes and interactions of molecules in solution

Viscometry



Light Scattering

Analytical Ultracentrifugation

Steve Harding, NCMH

University of Nottingham

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***

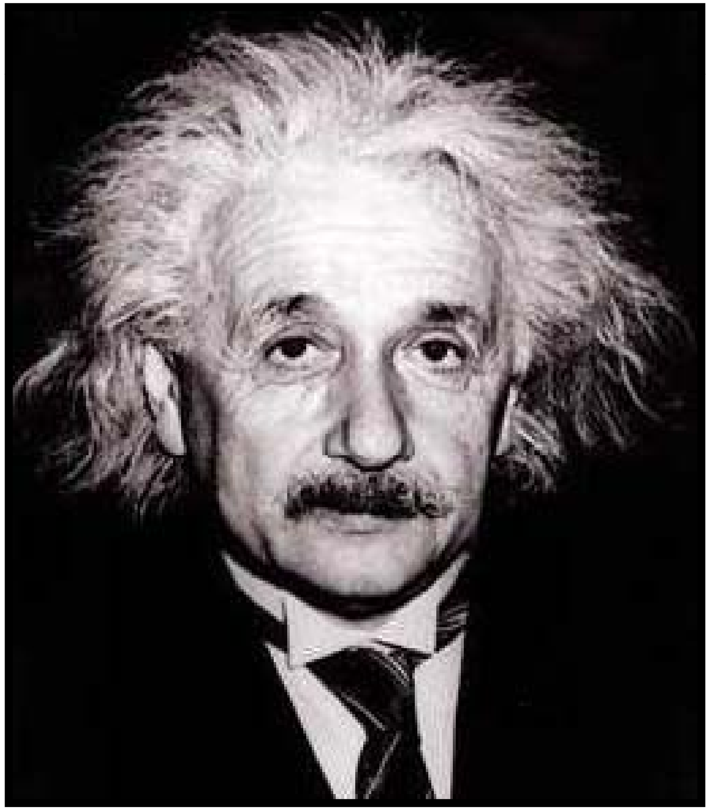
NCMH at Nottingham: A Facility for characterising sizes/shapes and interactions of macromolecules in solution

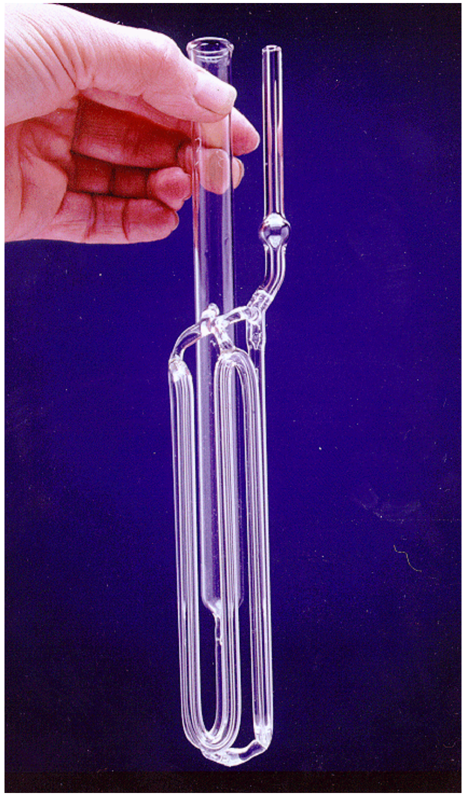
http://www.nottingham.ac.uk/ncmh


National Centre for
Macromolecular
Hydrodynamics
NCMH
University of
Nottingham

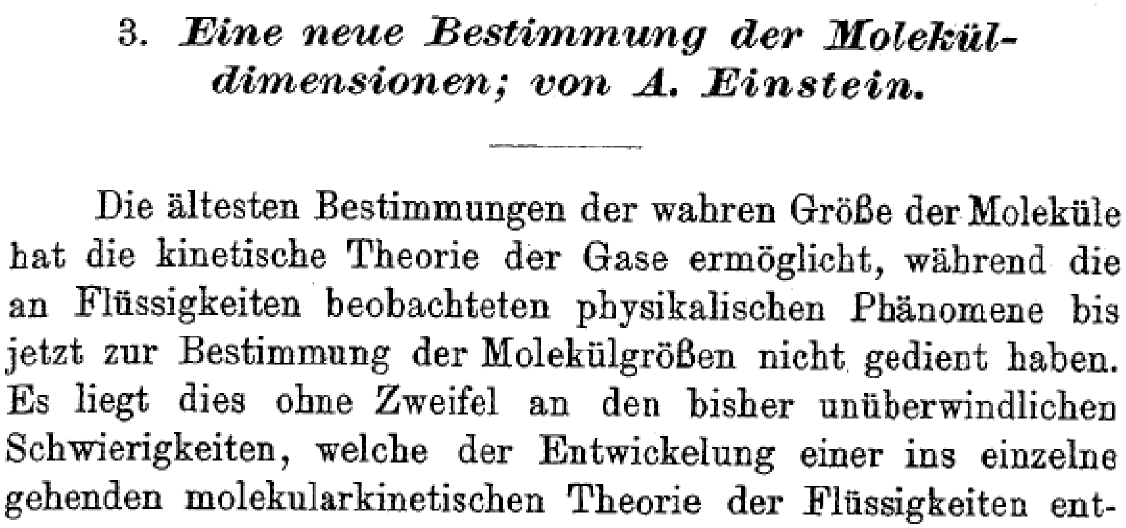
Lecture 1:

Albert Einstein and the Viscosity of Macromolecules





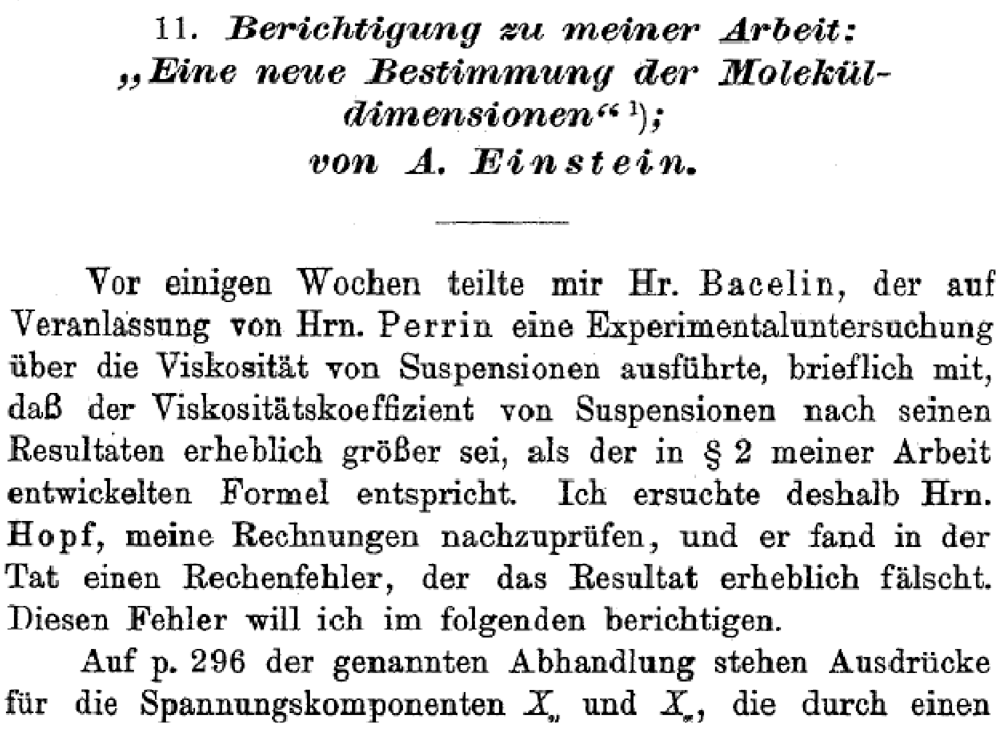
Annalen der Physik Band 19, 1906, 289-306:



3. *Eine neue Bestimmung der Moleküldimensionen; von A. Einstein.*

Die ältesten Bestimmungen der wahren Größe der Moleküle hat die kinetische Theorie der Gase ermöglicht, während die an Flüssigkeiten beobachteten physikalischen Phänomene bis jetzt zur Bestimmung der Molekülgrößen nicht gedient haben. Es liegt dies ohne Zweifel an den bisher unüberwindlichen Schwierigkeiten, welche der Entwickelung einer ins einzelne gehenden molekularkinetischen Theorie der Flüssigkeiten ent-

Annalen der Physik Band 34, 1911, 591-592:



11. *Berichtigung zu meiner Arbeit: „Eine neue Bestimmung der Moleküldimensionen“ [1]);*

von A. Einstein.

Vor einigen Wochen teilte mir Hr. Bacelin, der auf Veranlassung von Hrn. Perrin eine Experimentaluntersuchung über die Viskosität von Suspensionen ausführte, brieflich mit, daß der Viskositätskoeffizient von Suspensionen nach seinen Resultaten erheblich größer sei, als der in § 2 meiner Arbeit entwickelten Formel entspricht. Ich ersuchte deshalb Hrn. **Hopf**, meine Rechnungen nachzuprüfen, und er fand in der Tat einen Rechenfehler, der das Resultat erheblich fälscht. Diesen Fehler will ich im folgenden berichtigen.

Auf p. 296 der genannten Abhandlung stehen Ausdrücke für die Spannungskomponenten X_y und X_z, die durch einen

Viscometry

Intrinsic Viscosity of Macromolecular Solutions

Viscosity of biomolecules

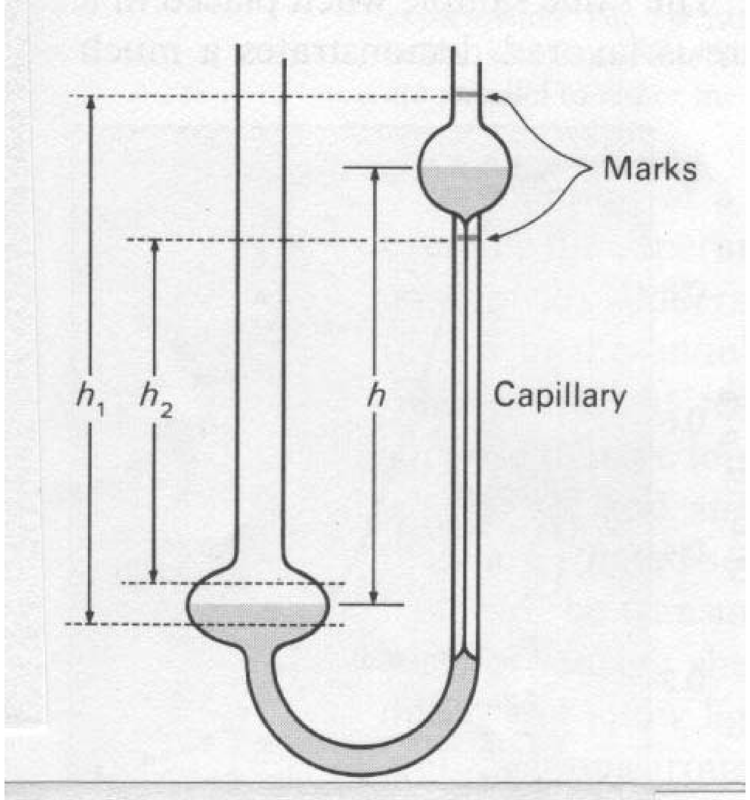
Why viscometry?

Simple, straightforward technique for assaying

1. Solution conformation of biomolecules & volume/ solvent association
2. Molecular weight of biomolecules
3. Flexibility " "

Types of Viscometer:

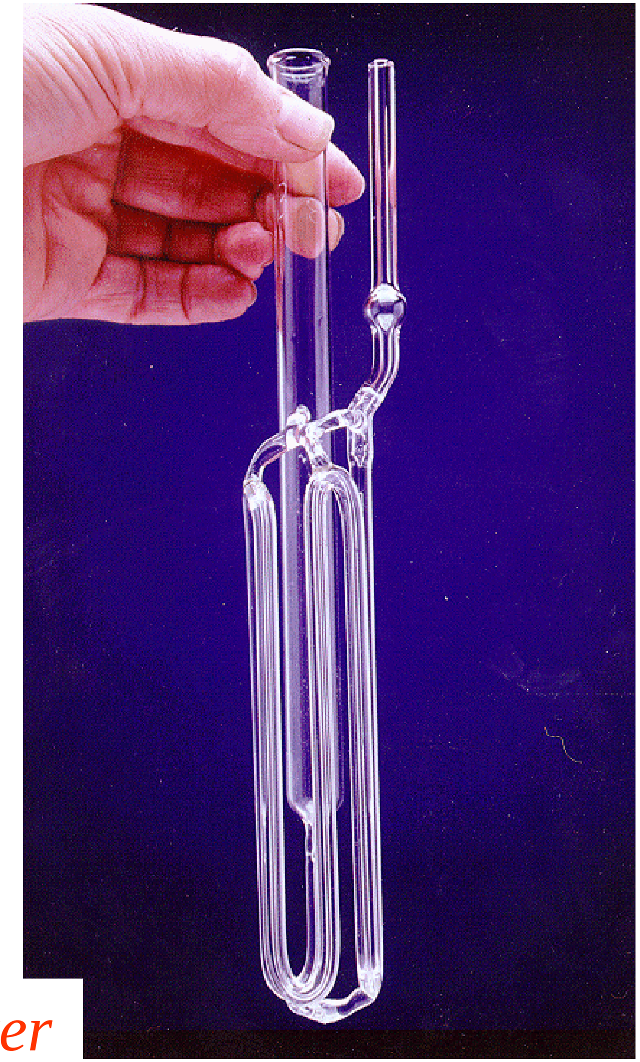
1. “U-tube” (Ostwald or Ubbelohde)
2. “Cone & Plate” (Couette)



Ostwald Viscometer

Types of Viscometer:

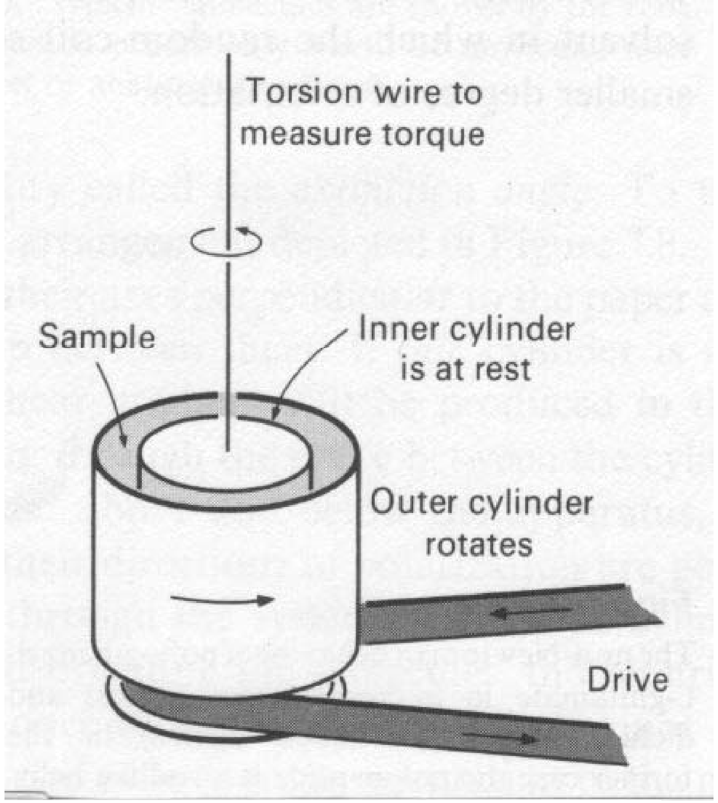
1. “U-tube” (Ostwald or Ubbelohde)
2. “Cone & Plate” (Couette)



Extended Ostwald Viscometer

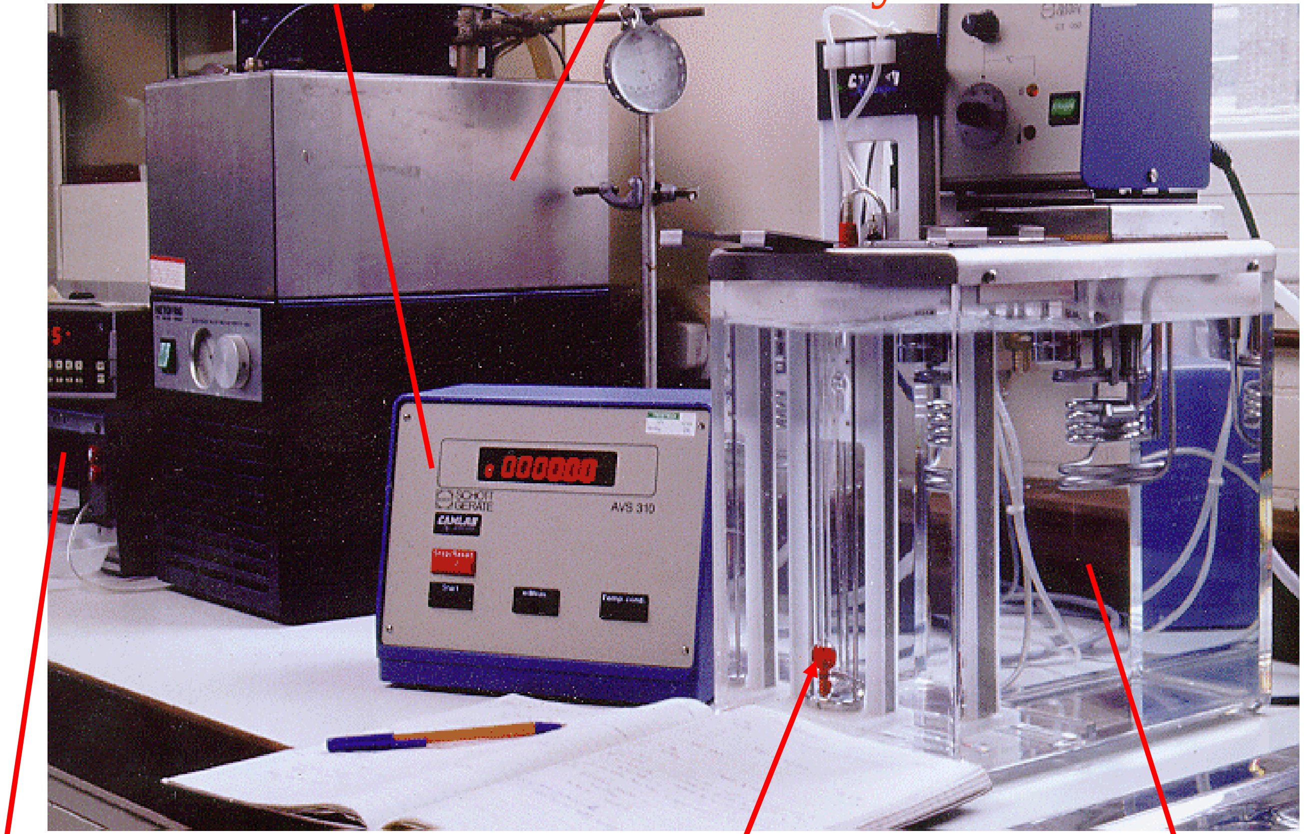
Types of Viscometer:

1. “U-tube” (Ostwald or Ubbelohde)
2. “Cone & Plate” (Couette)



Couette-type Viscometer

Anton-Paar AMVn Rolling Ball viscometer



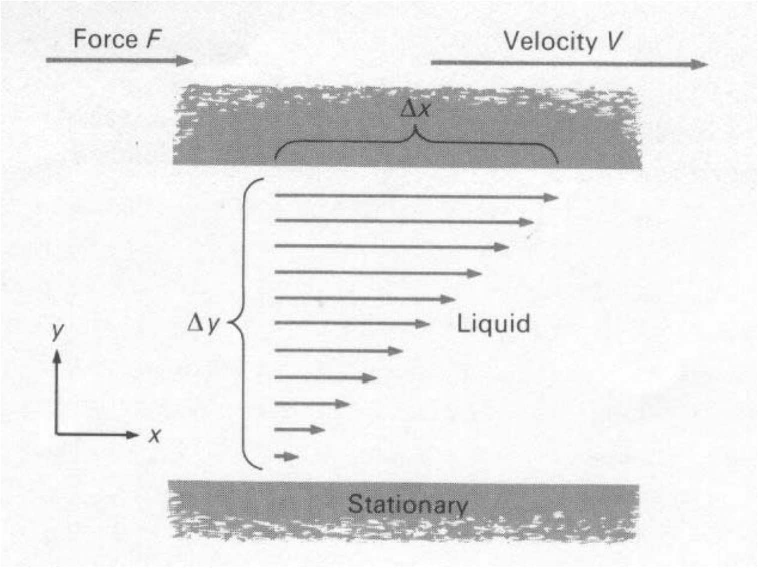
Auto-timer

Coolant system

Density meter

Solution

Water bath ±
0.01°C



Definition of viscosity:

For normal (Newtonian) flow

τ = (F/A) = η . (dv/dy)

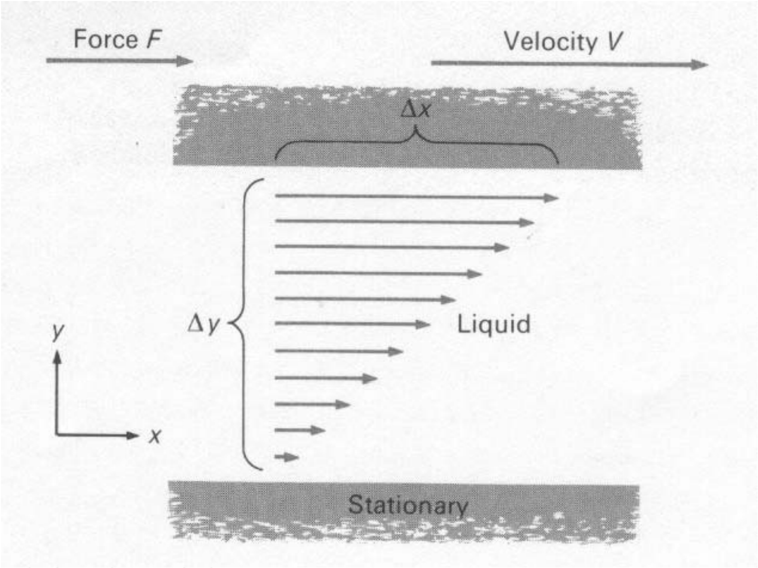
$$\boxed{\eta = \tau/(dv/dy)}$$

units: $(dyn/cm^2)/sec^{-1}$

$= dyn.sec.cm^{-2}$.

= <u>POISE</u> (P)

At 20.0^oC, $\eta(water) \sim 0.01P$



Definition of viscosity:

For normal (Newtonian) flow

$$\underbrace{\tau}_{\text{shear stress}} = (F/A) = \overbrace{\eta}^{\text{viscosity}} . \underbrace{(dv/dy)}_{\text{shear rate}}$$

$$\boxed{\eta = \tau/(dv/dy)}$$

units: $(\mathrm{dyn/cm^2})/\mathrm{sec}^{-1}$

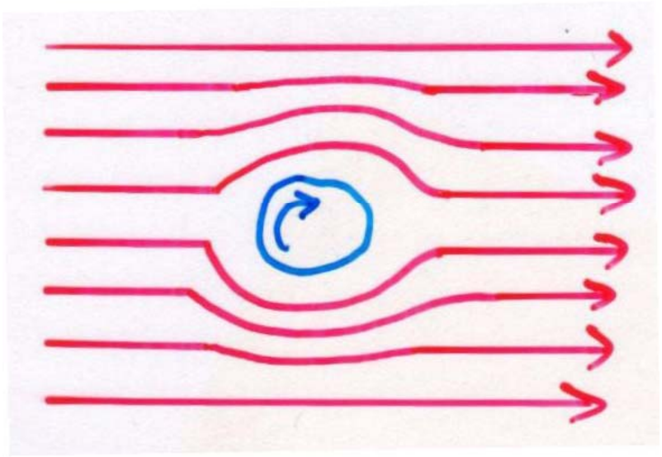
$= \mathrm{dyn.sec.cm^{-2}}$.

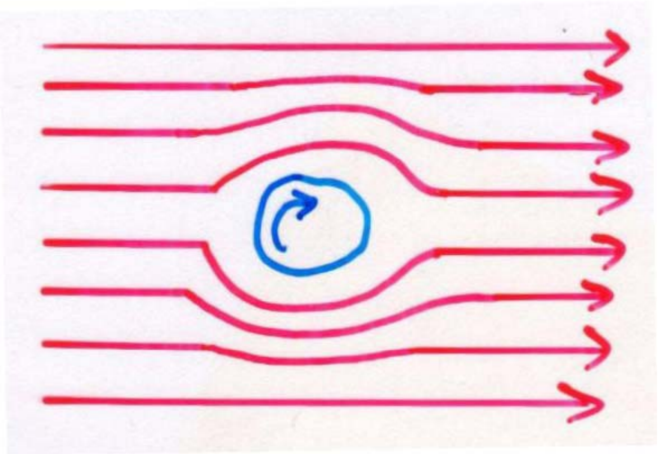
= <u>POISE</u> (P)

At 20.0°C, $\eta(\text{water}) \sim 0.01\text{P}$

Viscosity of biomolecular solutions:

A dissolved macromolecule will INCREASE the viscosity of a solution because it disrupts the streamlines of the flow:





We define the relative viscosity η_r as the ratio of the viscosity of the solution containing the macromolecule, η, to that of the pure solvent in the absence of macromolecule, η_o:

$\eta_r = \eta/\eta_o$ no units

For a U-tube viscometer, $\eta_r = (t/t_o).(\rho/\rho_o)$

Reduced viscosity

The relative viscosity depends (at a given temp.) on the concentration of macromolecule, the shape of the macromolecule & the volume it occupies.

If we are going to use viscosity to infer on the shape and volume of the macromolecule we need to eliminate the concentration contribution.

The first step is to define the reduced viscosity

$\eta_{red} = (\eta_r - 1)/c$

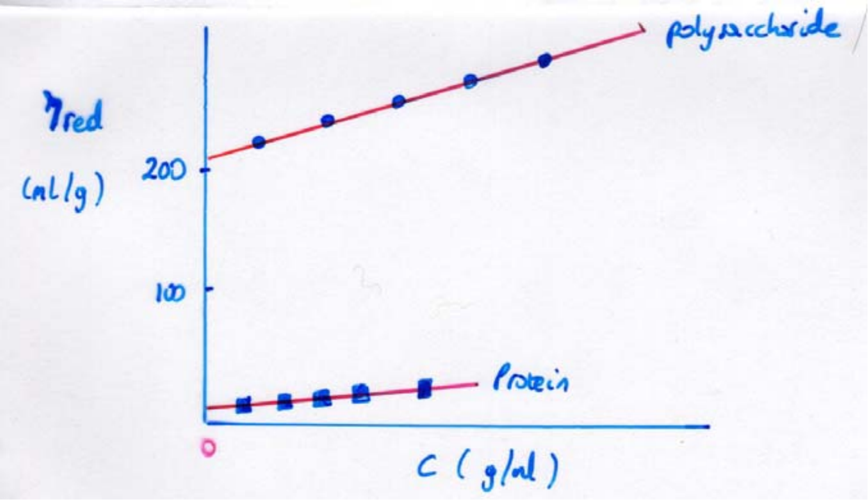
If c is in g/ml, units of η_{red} are ml/g

The Intrinsic Viscosity $[\eta]$

The next step is to eliminate non-ideality effects deriving from exclusion volume, backflow and charge effects. By analogy with osmotic pressure, we measure η_{red} at a series of concentrations and extrapolate to zero concentration:

$$\boxed{[\eta] = \mathrm{Lim}_{c \to 0} (\eta_{red})}$$

Units of $[\eta]$ are ml/g

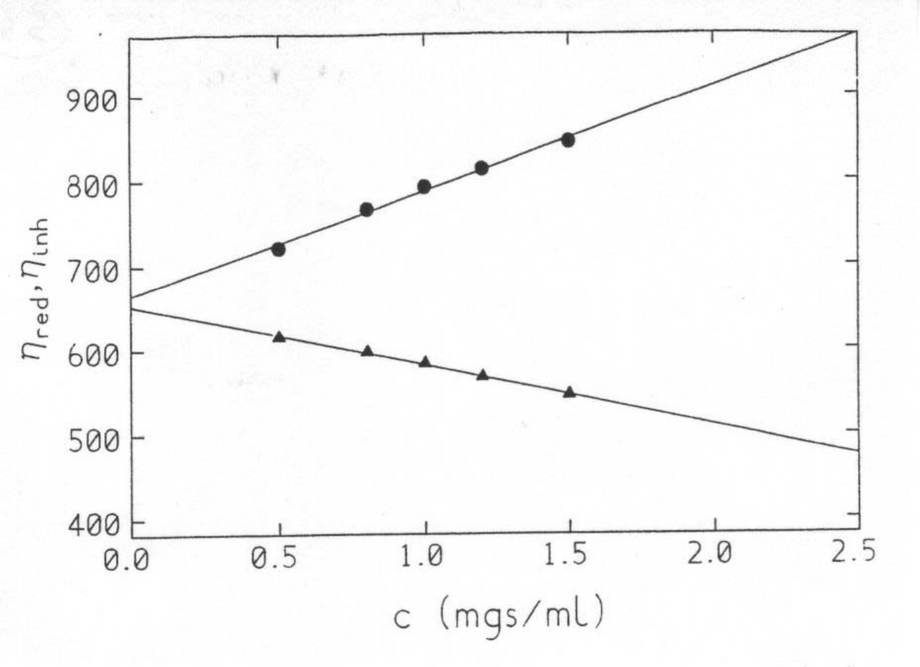


Form of the Concentration Extrapolation

2 main forms

Huggins equation: $\eta_{red} = [\eta]\,(1 + K_H[\eta]c)$

Kraemer equation: $(\ln\eta_r)/c = [\eta]\,(1 - K_K[\eta]c)$



K_H (no units):
HUGGINS
CONSTANT

K_K (no units):
KRAEMER
CONSTANT

A variant of the Huggins equation is:

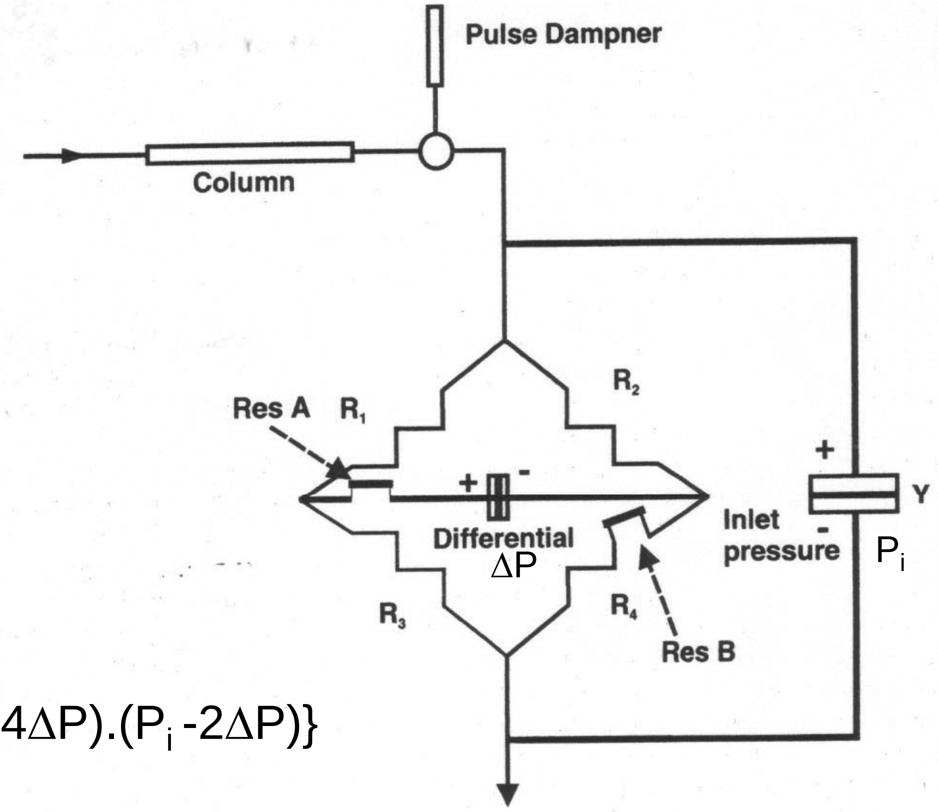
$$\eta_{red} = [\eta] (1 + k_{\eta}.c) \qquad k_{\eta}: \mathrm{ml/g}$$

and another important relation is the SOLOMON-CIUTA relation, essentially a combination of the Huggins and Kraemer lines:

$$[\eta] \sim (1/c) . [2 (\eta_r - 1) - 2 \ln(\eta_r)]^{1/2}$$

The Solomon-Ciuta equation permits the approximate evaluation of $[\eta]$ without a concentration extrapolation.

Differential Pressure Viscometer:



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

Intrinsic Viscosity and its relation to macromolecular properties

$[\eta]$ so found depends on the shape, flexibility and degree of (time-averaged) water-binding, and for non-spherical particles the molecular weight:

	M (g/mol)	[η] (ml/g)	
Glucose	180	3.8	**GLOBULAR**
Myoglobin	17000	3.25	
Ovalbumin	45000	3.49	
Hemoglobin	68000	3.6	
Soya-bean 11S	350000		
Tomato bushy stunt virus	10.7 x 10^6	3.4	
Fibrinogen	330000	27	**RODS, COILS**
Myosin	490000	217	
Alginate	200000	700	

Intrinsic Viscosity and Protein Shape and Hydration

$$[\eta] = \nu \,.\, v_s \qquad (1)$$

ν: Simha-Saito function (function of shape & flexibility)
v_s: swollen specific volume, ml/g (function of H_2O interaction)

ν: Einstein value of 2.5 for rigid spheres
>2.5 for other shapes

v_s: volume of “hydrated” or “swollen” macromolecule per unit anhydrous mass

$$= \bar{v} + (\delta/\rho_o) \quad = \bar{v} \,.\, S_w$$

δ: “hydration” (g H_2O/g protein)

$\bar{v}$: partial specific volume (anhydrous volume per unit anhydrous mass)

So, 3 forms of Eqn. (1):

$$[\eta] = \nu \, . \, v_s$$

or

$$[\eta] = \nu \, . \, \{\bar{v} + (\delta/\rho_o)\}$$

or

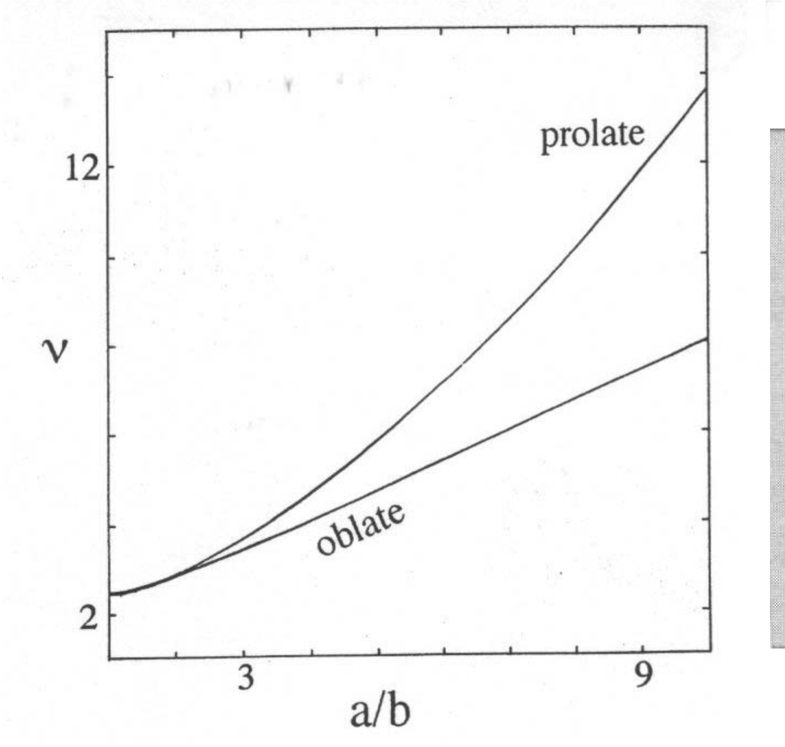
$$[\eta] = \nu \, . \, \bar{v} \, . \, S_w$$

For proteins, $\bar{v} \sim 0.73$ml/g, $v_s \sim 1$ml/g, $S_w \sim 1.4$,

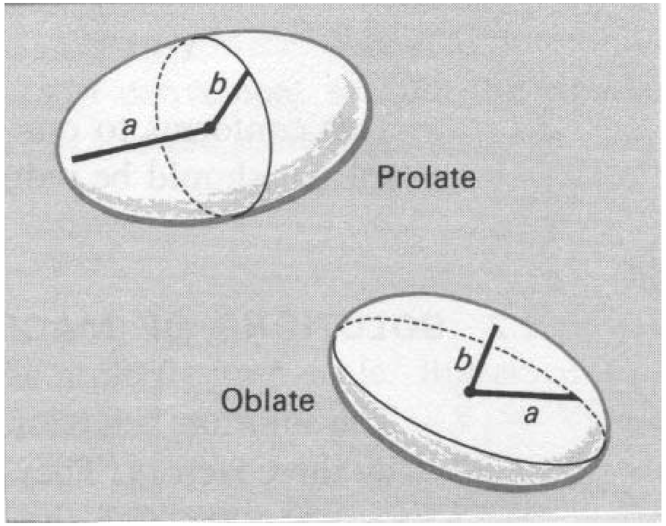
{For polysacchs, $\bar{v} \sim 0.6$ml/g, $v_s >> 1$ml/g, $S_w >> 1$}

Getting a shape from the viscosity ν parameter

SIMPLE ELLIPSOIDS OF REVOLUTION:

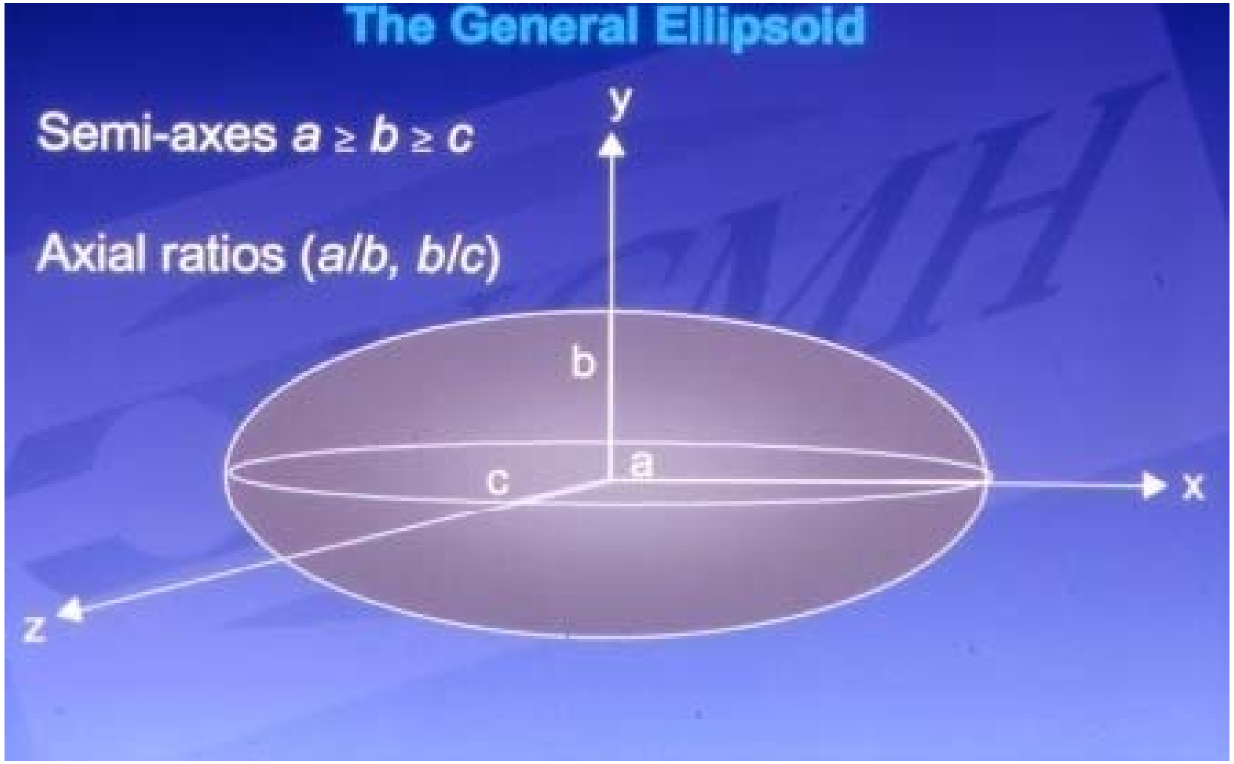


axial ratio: a/b



Computer program ELLIPS1 downloadable from www.nottingham.ac.uk/ncmh

Getting a shape from the viscosity ν parameter



The General Ellipsoid

Semi-axes $a \geq b \geq c$

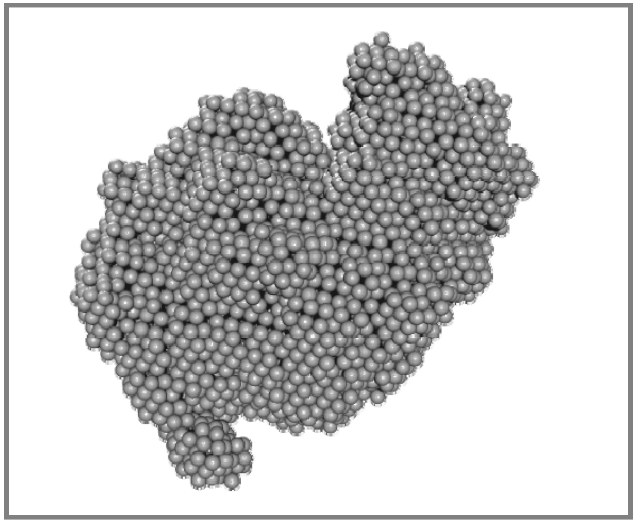
Axial ratios $(a/b, b/c)$

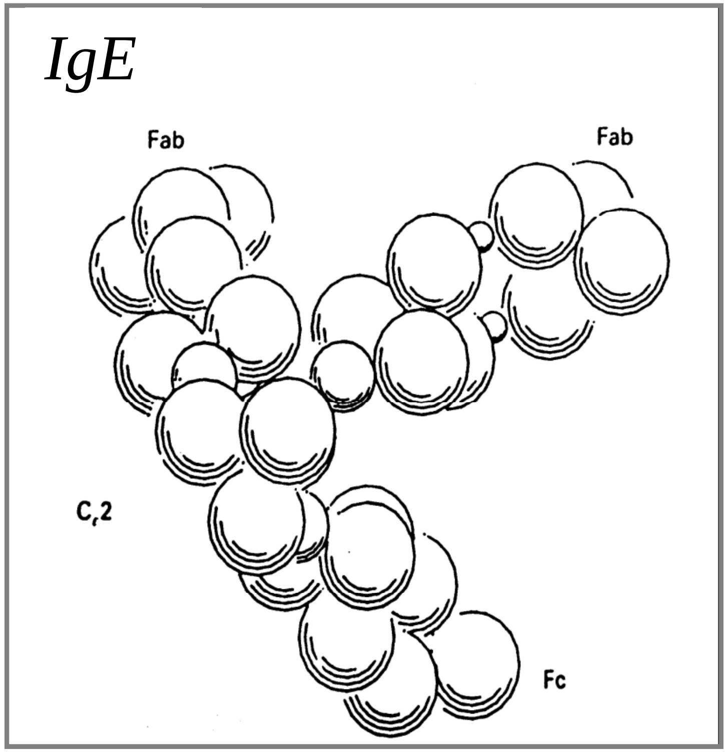
Computer program ELLIPS2 downloadable from www.nottingham.ac.uk/ncmh

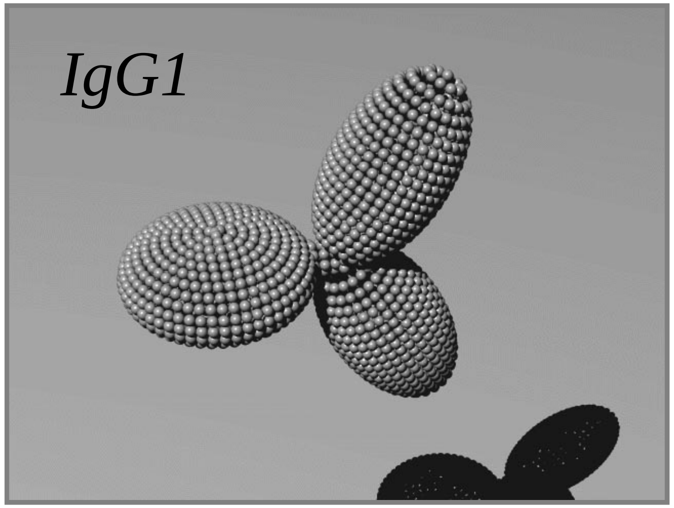
For more complicated shapes:

BEAD & SHELL MODELS

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

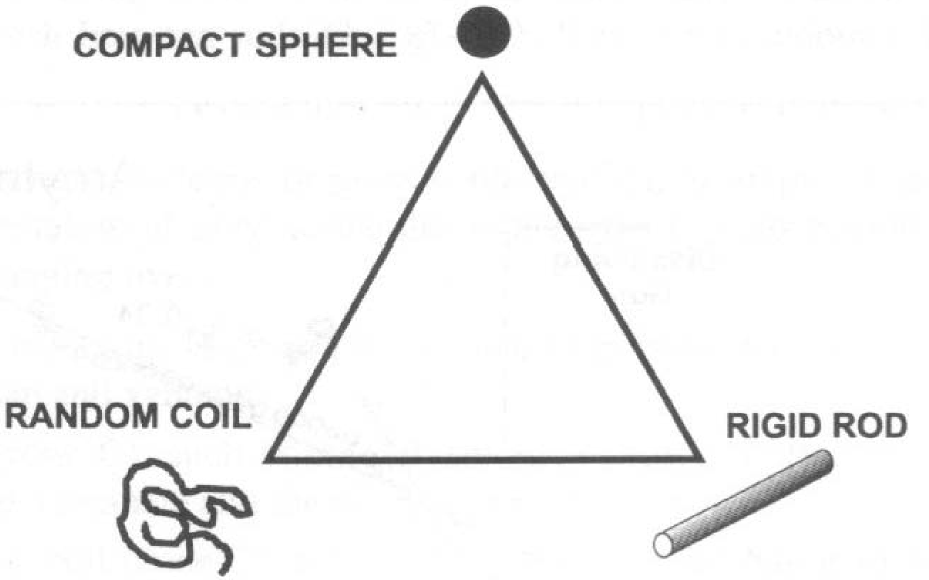






GENERAL CONFORMATIONS

The three extremes of macromolecular conformation (COMPACT SPHERE, RIGID ROD, RANDOM COIL) are conveniently represented at the corners of a triangle, known as the HAUG TRIANGLE:



Each extreme has its own characteristic dependence of $[\eta]$ on M.

Mark-Houwink-Kuhn-Sakurada equation

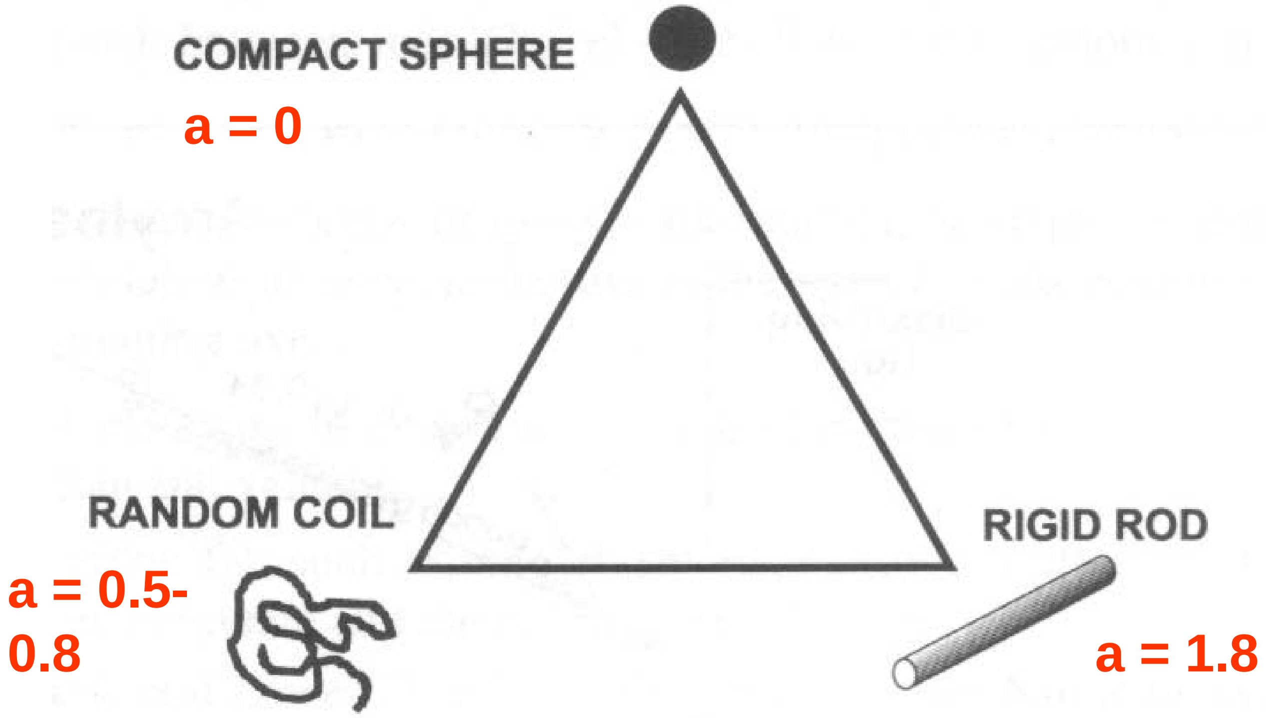
$$[\eta] = K'.M^{a}$$

Analagous power law relations exist for sedimentation, diffusion and R_g (classical light scattering)

$$s^{o}_{20,w} = K''.M^{b}; \quad D^{o}_{20,w} = K'''.M^{-\varepsilon}; \quad R_g = K''''.M^{c};$$

By determining a (or b, ε or c) for a homologous series of a biomolecule, we can pinpoint the conformation type

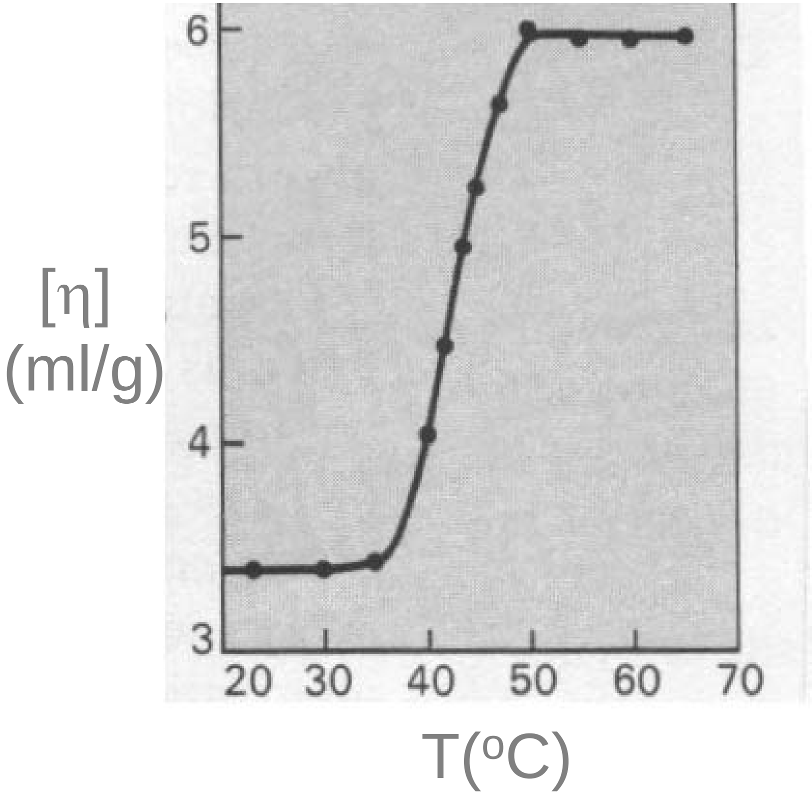
$$[\eta] = K'.M^a$$



Globular proteins, a~0.0, polysaccharide, a ~ 0.5 – 1.3

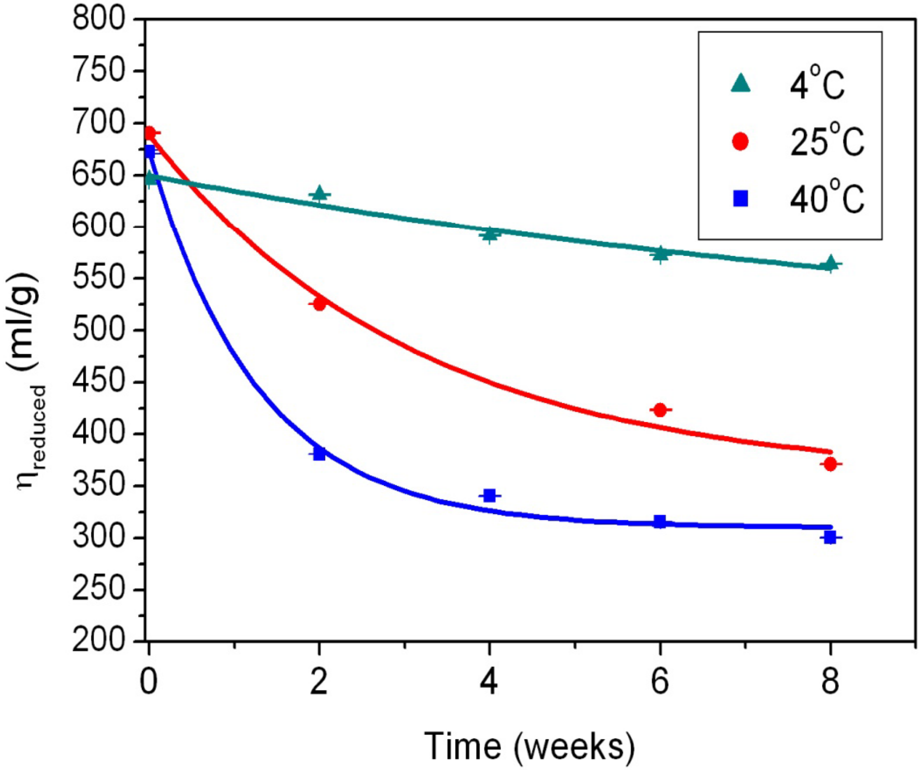
The intrinsic viscosity is ideal for monitoring conformation change:

Denaturation of ribonuclease



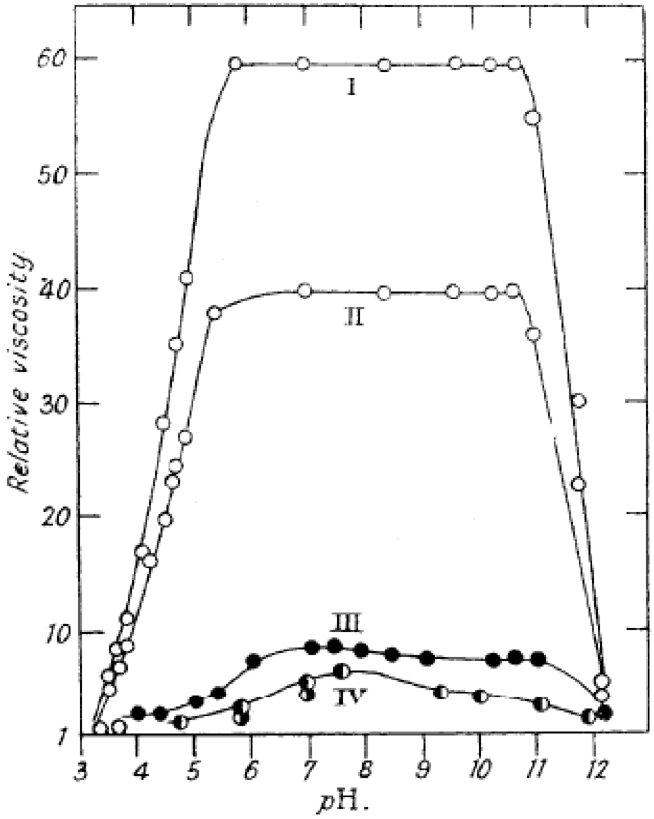
The intrinsic viscosity is also ideal for monitoring stability:

Storage of chitosan (used in nasal drug delivery)



Fee et al, 2006

Demonstration of H-bonding in DNA



Creeth, J.M., Gulland J.M. & Jordan, D.O. (1947) J. Chem. Soc. 1141-1145

The variation of the viscosity of solutions of various specimens of deoxypentose nucleic acid.

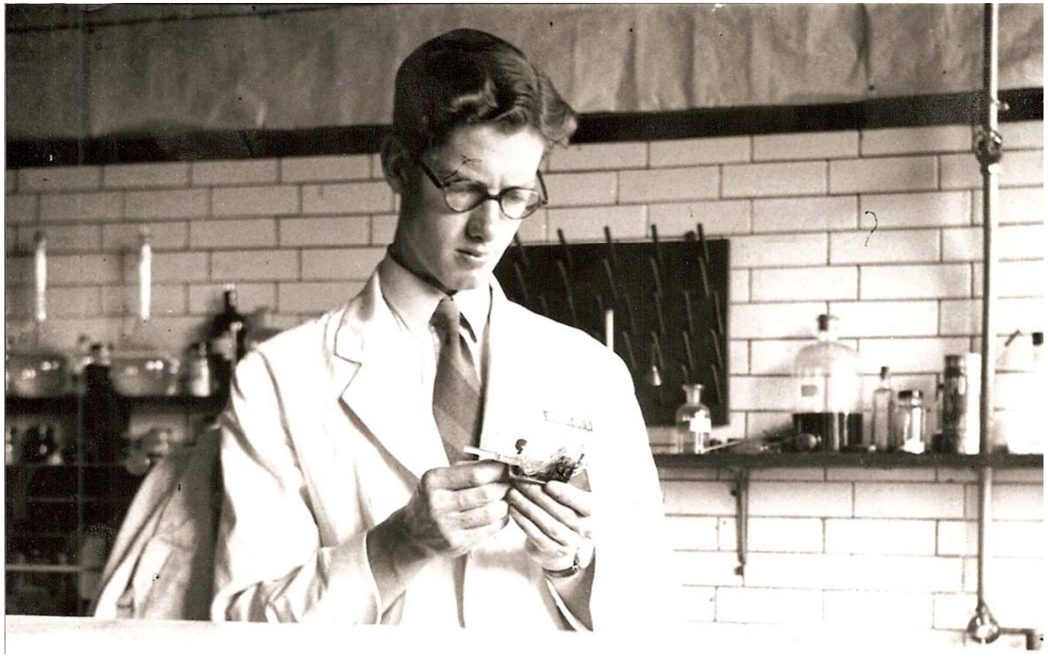
Tetrasodium salt of deoxypentose nucleic acid of calf thymus, ○ *:*

I (*applied pressure* 3000 *dynes/cm.*^{2}), II (*applied pressure* 7000 *dynes/cm.*^{2}).

Tetrasodium salt of deoxypentose nucleic acid after alkaline treatment, III, ● *; after acid treatment,* IV, ◐.

Tetrasodium salt of deoxypentose nucleic acid of calf thymus supplied by Professor Caspersson, IV, ◑.

J.Michael Creeth, 1924-2010



Follow up reference sources:

Serydyuk, I.N., Zaccai, N.R. and Zaccai, J. (2006) *Methods in Molecular Biophysics*, Cambridge, Chapter D9

Harding, S.E. (1997) "The intrinsic viscosity of biological macromolecules. Progress in measurement, interpretation and application to structure in dilute solution" *Prog. Biophys. Mol. Biol* **68,** 207-262. http://www.nottingham.ac.uk/ncmh/harding_pdfs/Paper192.pdf

Tombs, M.P. and Harding, S.E. (1997) *An Introduction to Polysaccharide Biotechnology*, Taylor & Francis, ISBN 0-78074-405169