STEP ITN Lectures

Marie Curie Initial Training Network

Shaping and Transformation in the Engineering of Polysaccharides (STEP)

Steve Harding, NCMH

University of Nottingham


National Centre for
Macromolecular
Hydrodynamics
NCMH


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Hydrodynamics

STEP ITN Lectures

September 2009: Hydrodynamic characterisation of macromolecules

http://www.stepitn.eu/?page_id=1113

February 2010: Sizes, shapes & interactions of molecules in solution

- Albert Einstein and the Viscosity of Macromolecules
- Light Scattering and SEC-MALLs
- Dynamic Light Scattering
- Analytical Ultracentrifugation I
- Analytical Ultracentrifugation II: Interactions

http://www.stepitn.eu/?page_id=1137

June-July 2010: From sticky mucus to probing our past: Aspects and problems of the Biotechnological use of Macromolecules

http://www.chemie.uni-jena.de/institute/oc/heinze/Lecture_harding.html

From Sticky Mucus to Probing our Past: Aspects and problems of the Biotechnological use of Macromolecules

Datum/Zeit	Veranstaltungsort	Thema
Mi, 30.06.2010 12.15-13.45	SR 309 Carl-Zeiss-Str. 3	***Macromolecules as BioPharma mucoadhesives***
Do, 01.07.2010 08.15-09.45	SR 308 Carl-Zeiss-Str. 3	***Macromolecules as vaccines***
Do, 01.07.2010 13.15-14.45	HS Haus 1 August-Bebel-Str. 2	***Stability in response to Bioprocessing I. Thermal Processing, D, z and F values***
Fr, 02.07.2010 08.15-09.45	HS Haus 1 August-Bebel-Str. 2	***Stability in response to Bioprocessing II: Irradiation and freezing***
Fr, 02.07.2010 12.15-13.45	SR 307 Carl-Zeiss-Str. 3	***The use of non-recombining parts of the Y-chromosomal DNA and mitochondrial DNA as a probe into our past***

From Sticky Mucus to Probing our Past: Aspects and problems of the Biotechnological use of Macromolecules

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Macromolecules as BioPharma mucoadhesives

Steve Harding

The University of Nottingham

STICKY MUCUS IS IMPORTANT!

Consider a typical animal, zum beispiel - a Slug:

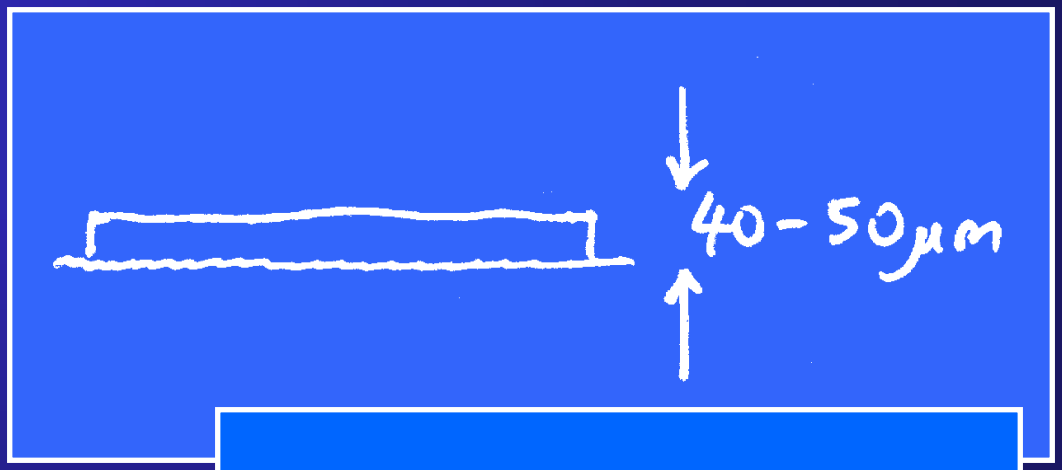
“If its love that makes the world go round”

“If its love that makes the world go round”

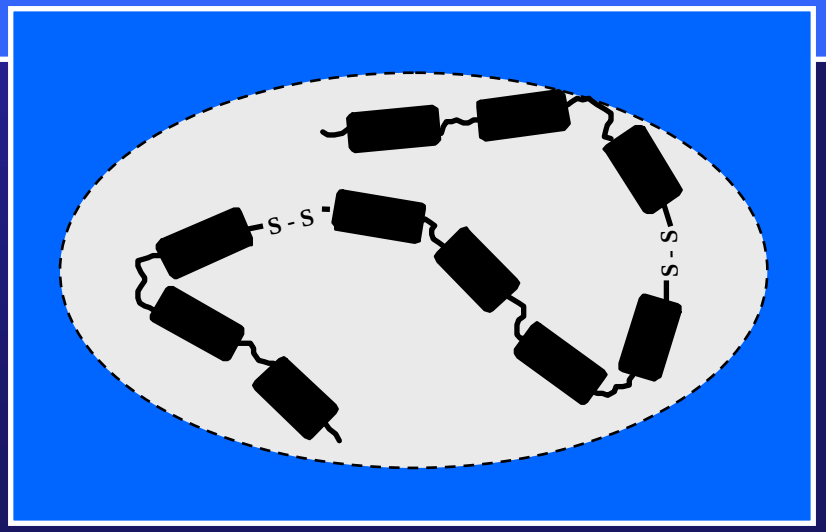
“then its mucus and slime which keeps it in perpetual motion”

Mucus

(i) adherent mucus gel in human gi tract

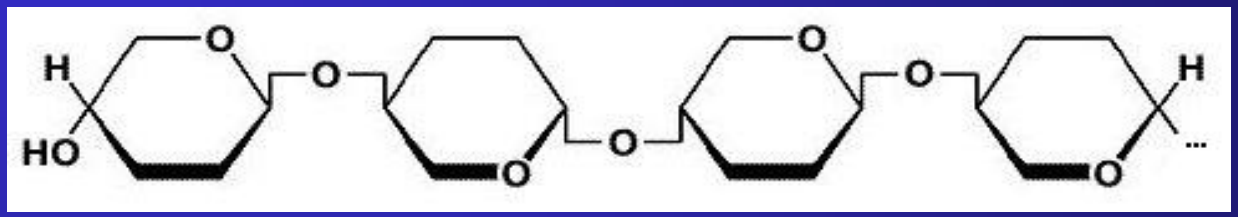


(ii) mucin glycoprotein

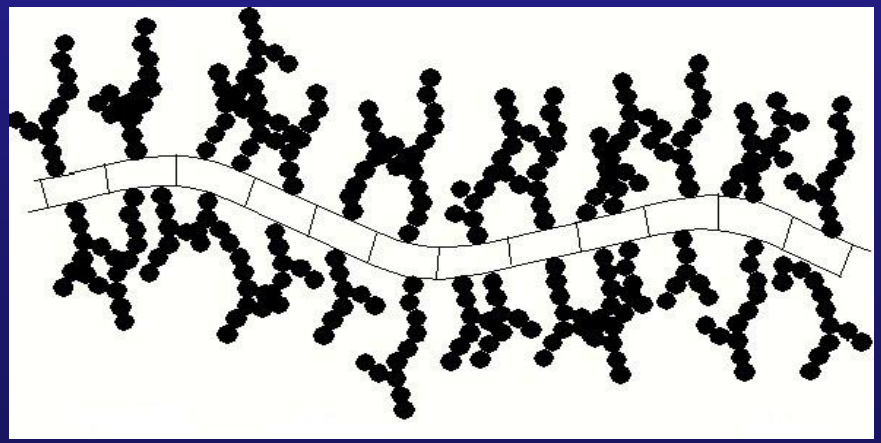


Pig colonic mucin: Jumel et al, 1997

Polysaccharides – from jam, jellies,



Mucus Glycoproteins – "mucins"



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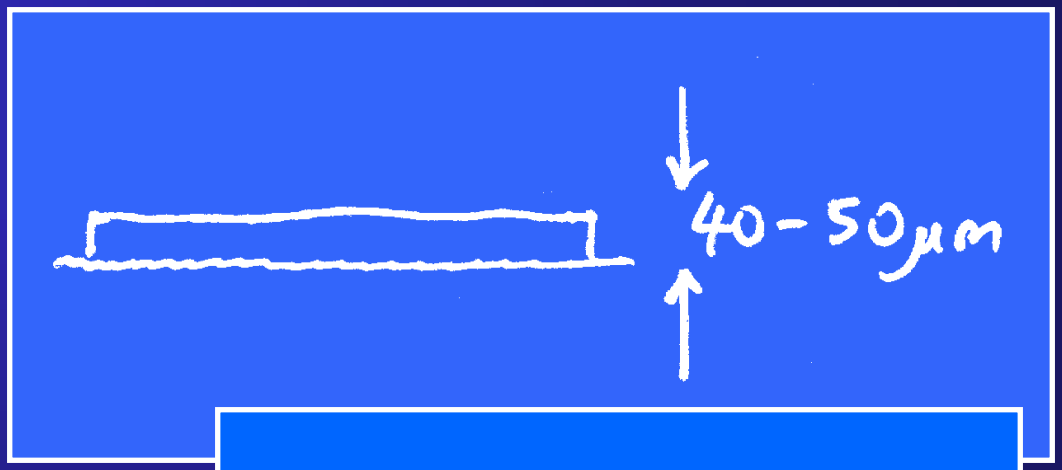
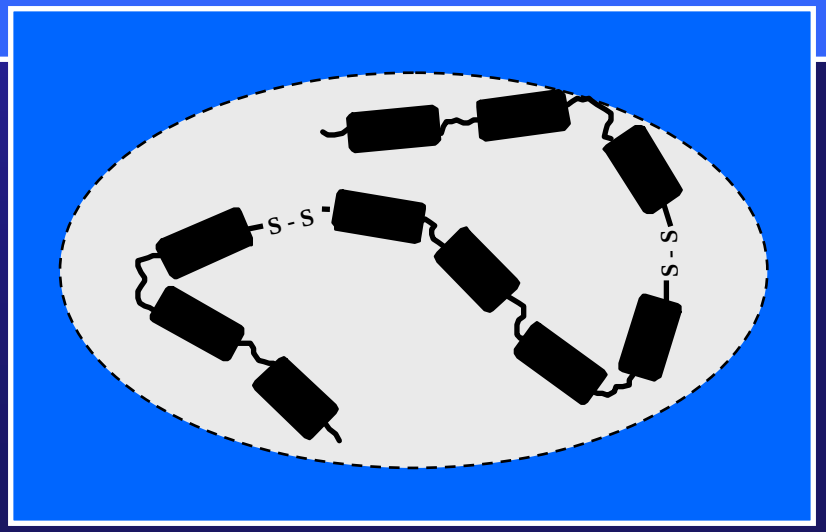
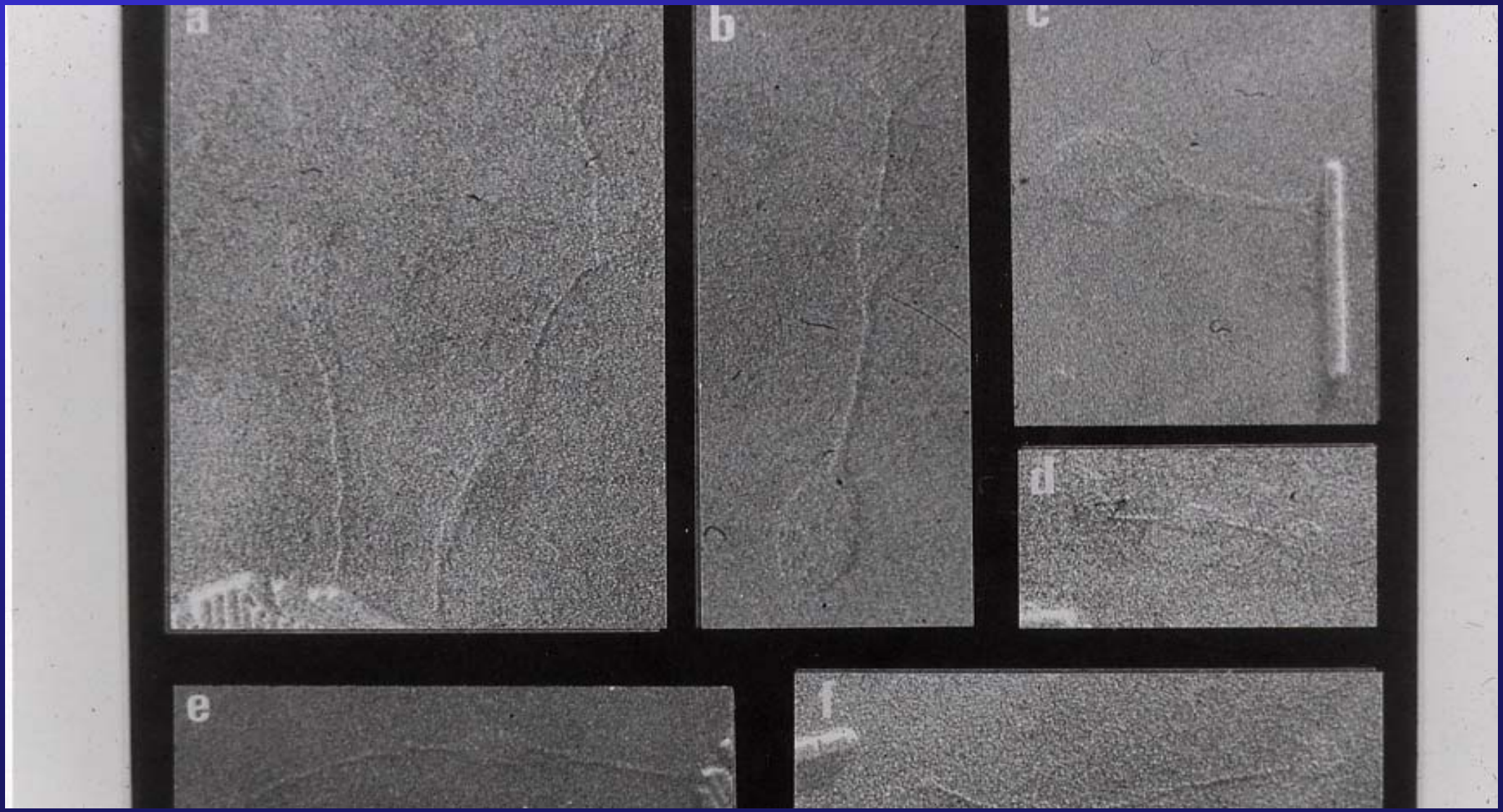


(ii) mucin glycoprotein

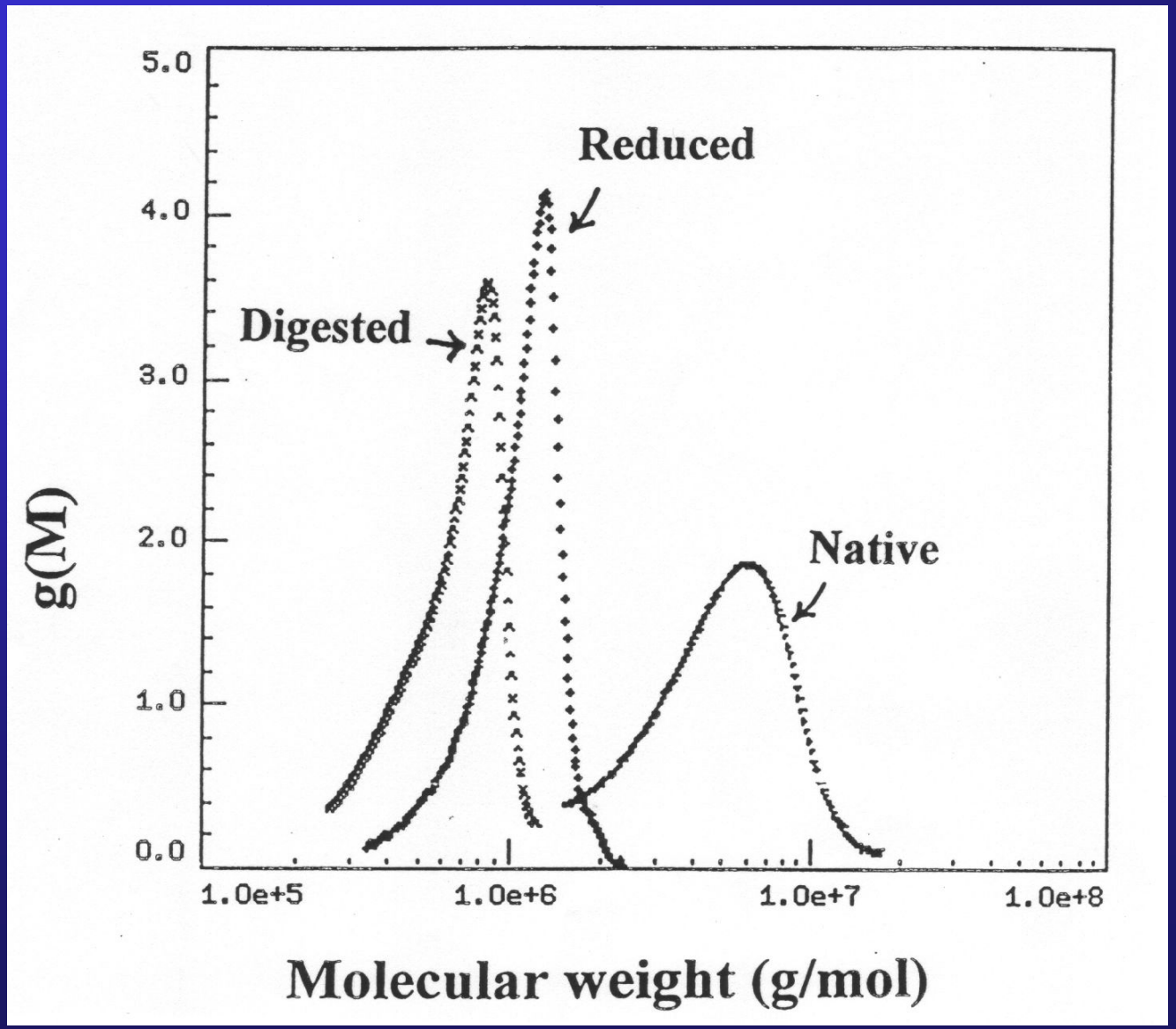


Pig colonic mucin: Jumel et al, 1997

Electron microscopy of bronchial mucins. Harding, Rowe and Creeth, 1983



Mucins have a very broad molecular weight distribution…



Jumel et al, 1997

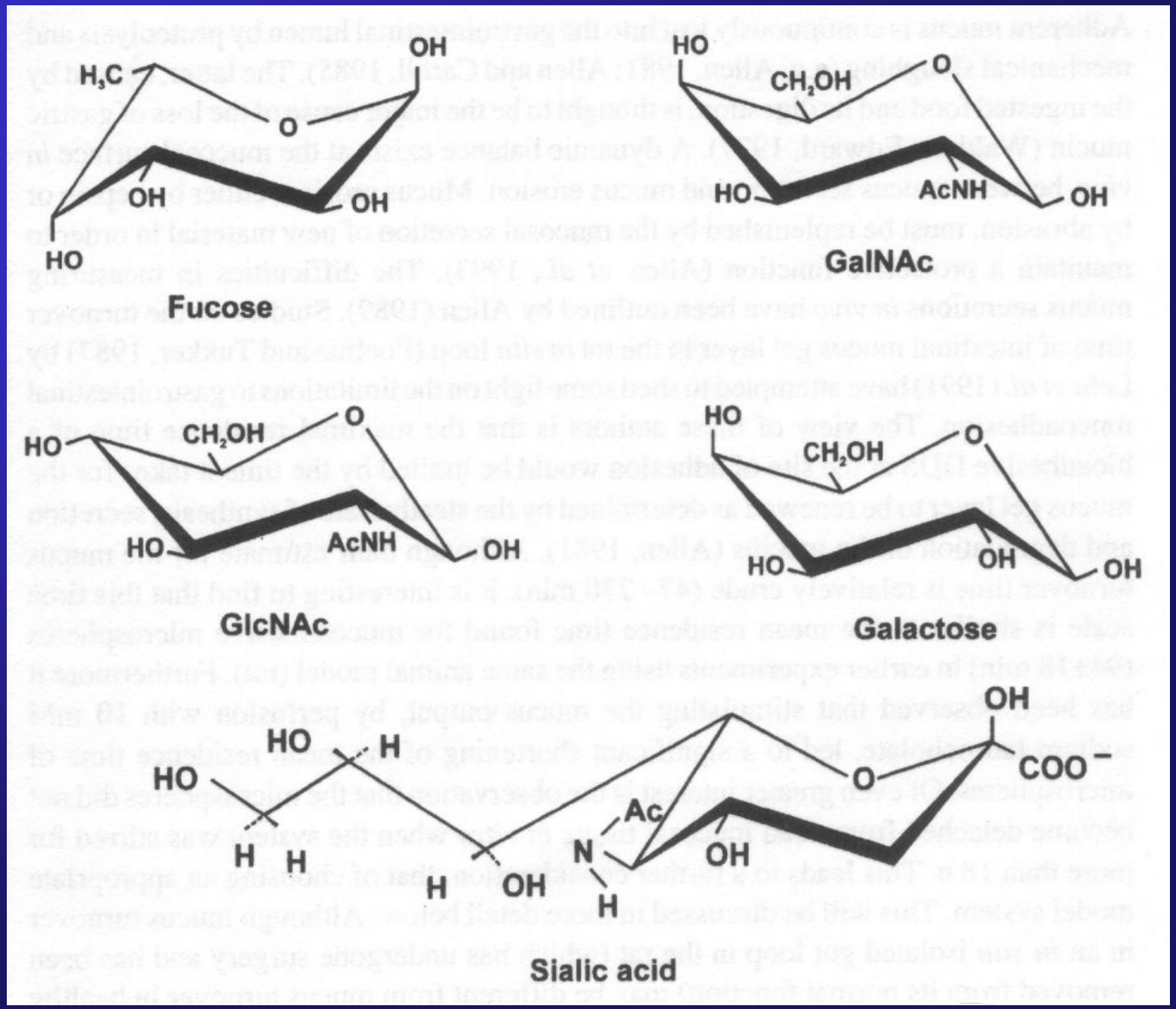
Mucin types

Hounsell, E.F., 2000

Mucin	Main expression	Chromosome	Amino acids in Tandem repeat*	Does it gel?
MUC1	Breast, Pancreas	1	20	NO
MUC2	Intestine, Tracheobronchus	11	23*	YES
MUC3	Intestine, gall	7	7	YES
MUC4	Colon, Tracheo,	3	16	YES
MUC5A/C	Cervix	11	8	YES
MUC5B	Stomach, Tracheo, Cervix, Eye	11	29	YES
MUC6	Tracheo, Salivary	11	169	YES
MUC7	Stomach, gallbladder	4	23	NO
MUC8	Salivary	12	41	YES

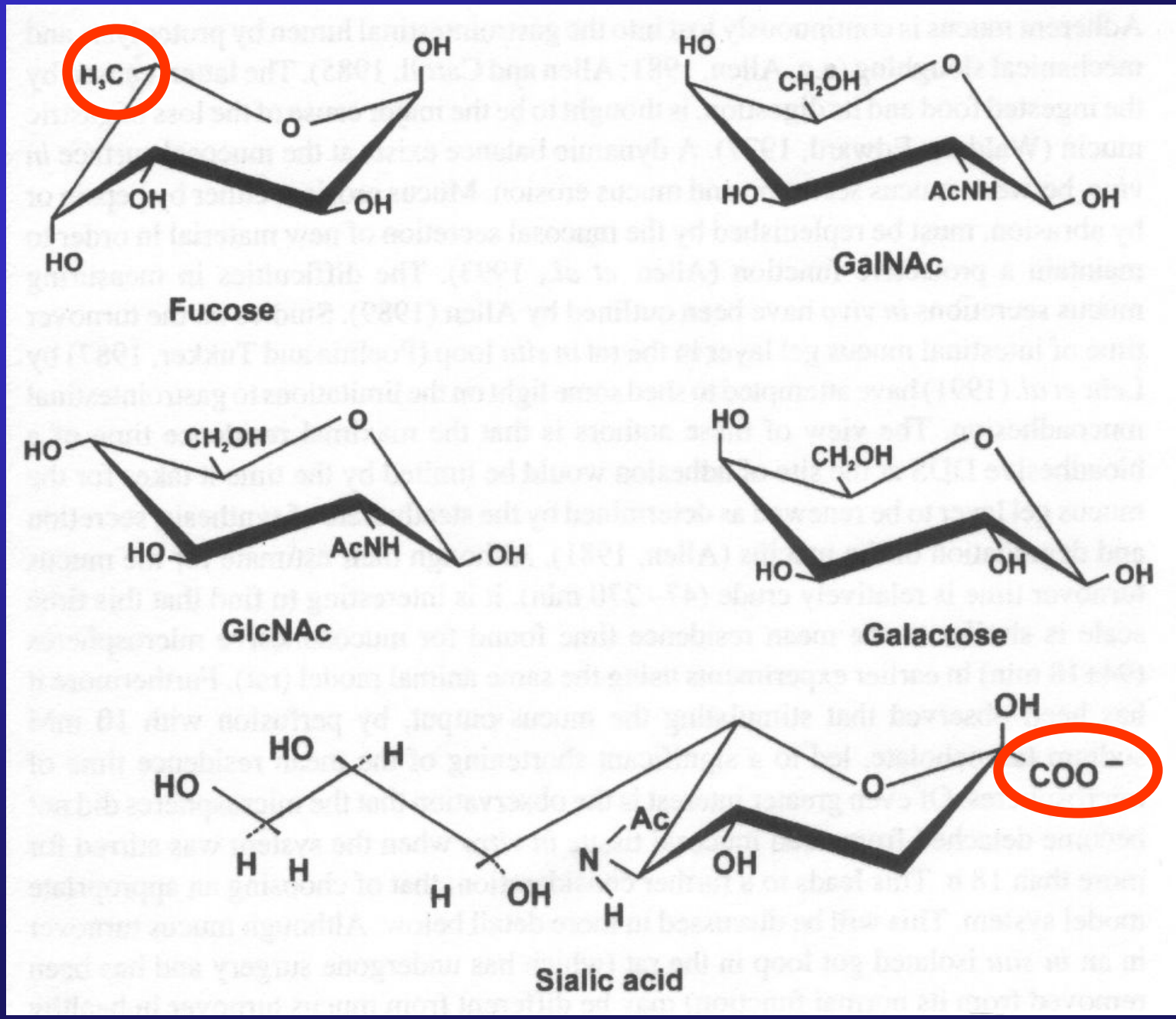
*MUC 2 tandem repeat: PTTPITTTTVTPTPTPTGTQT. MUC3: HSTPSFTSSITTETTS.
MUC4: TSSASTGHATPLPVTD; MUC5A/C: TTSTTSAP There are ~ 17 MUC genes now identified

Mucin sugars



Mucin sugars

Sticky bits



Mucin sugars

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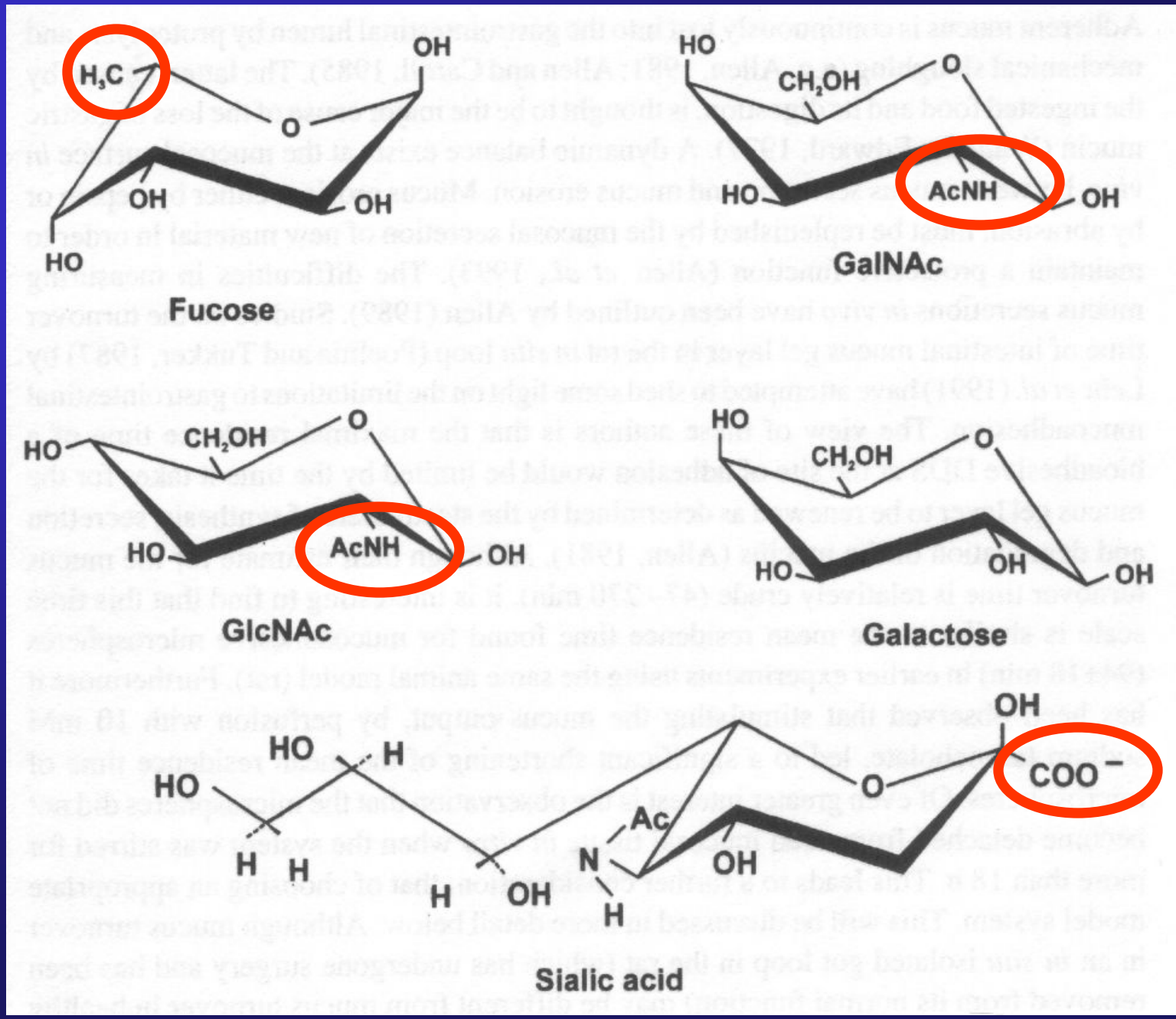

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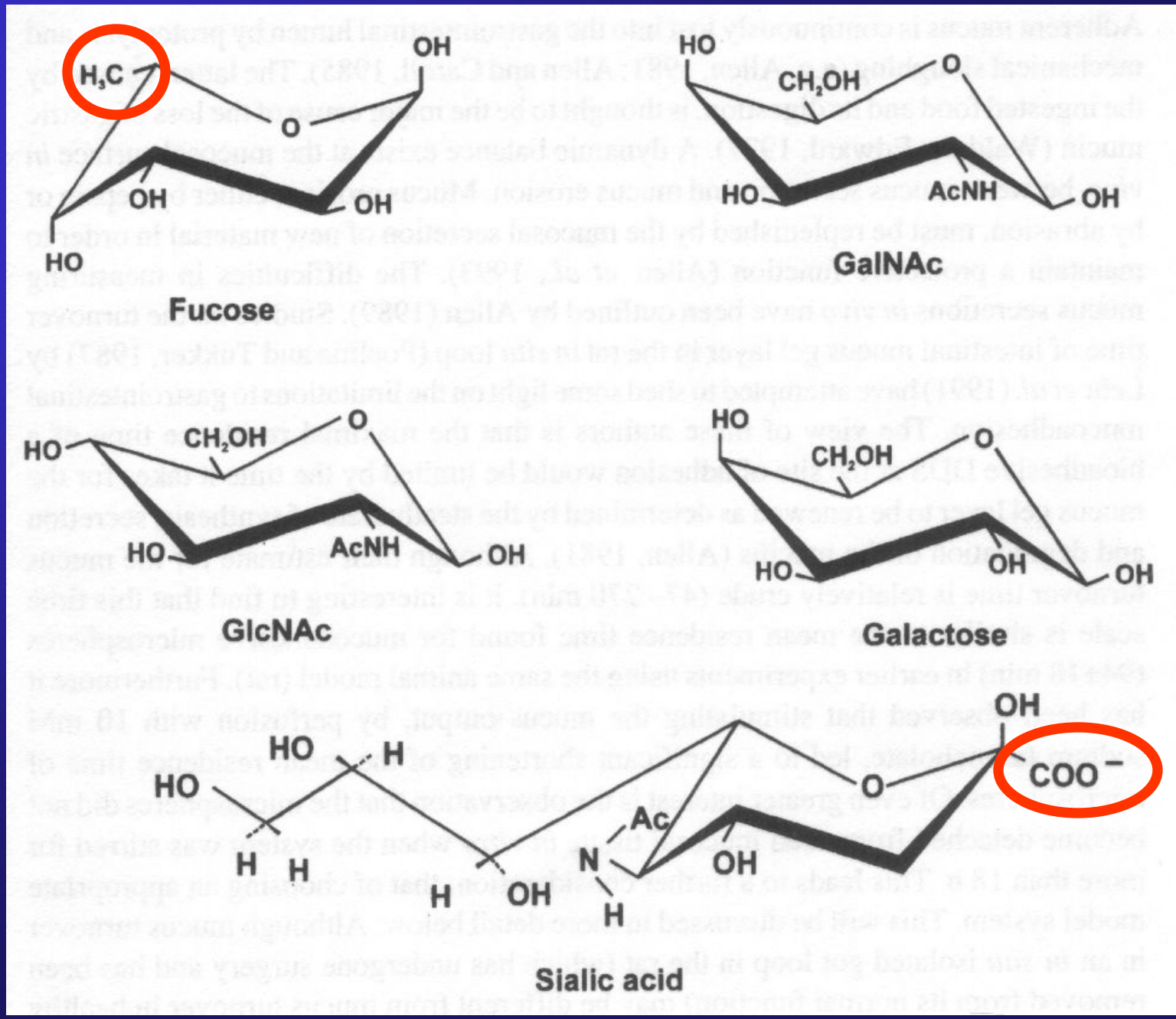
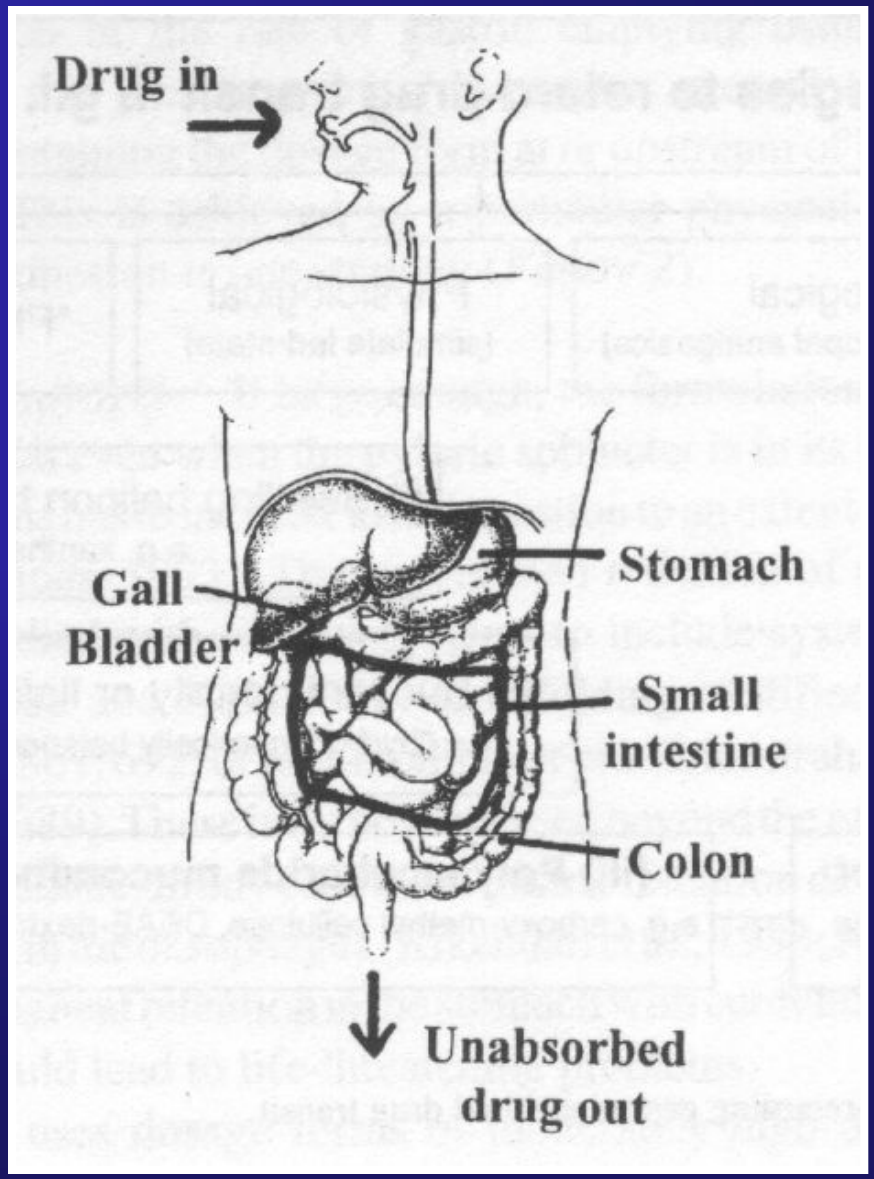


So, mucins are:

1. Large, hydrated, polydisperse, flexible coil
2. 80-90% glycosylated: key sites for interaction on sugars
3. Electrostatic sites: sialic acid (and also possible sulphonated groups)
4. Hydrophobic: fucose

Oral drug administration

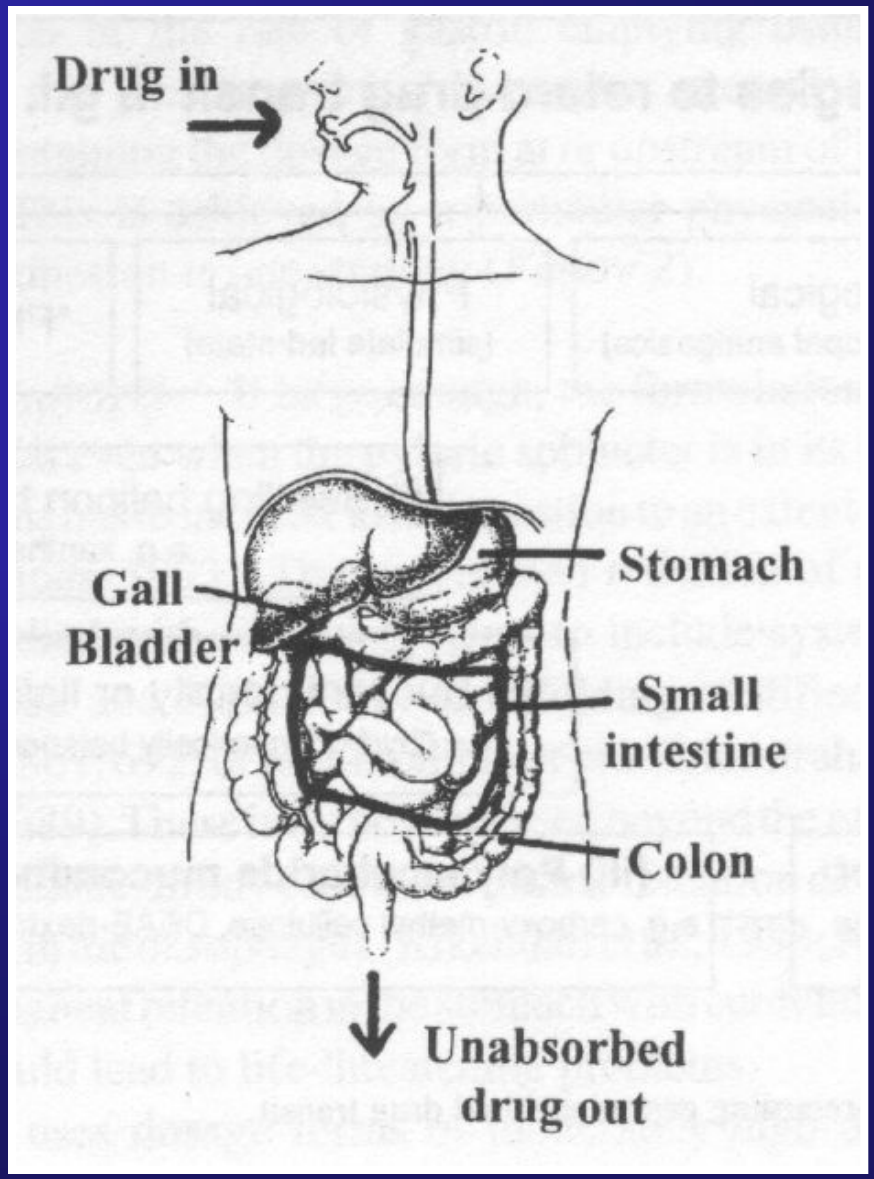
- most popular with medical staff & patients
- majority of drug absorbed at small intestine(~100m^2)
- clearance time though generally too short (4-12h)



Oral drug administration

Low appearance of drug due to

- too rapid a transit past the ideal absorption site
- rapid degradation in the g.i. tract once released
- low transmucosal permeability

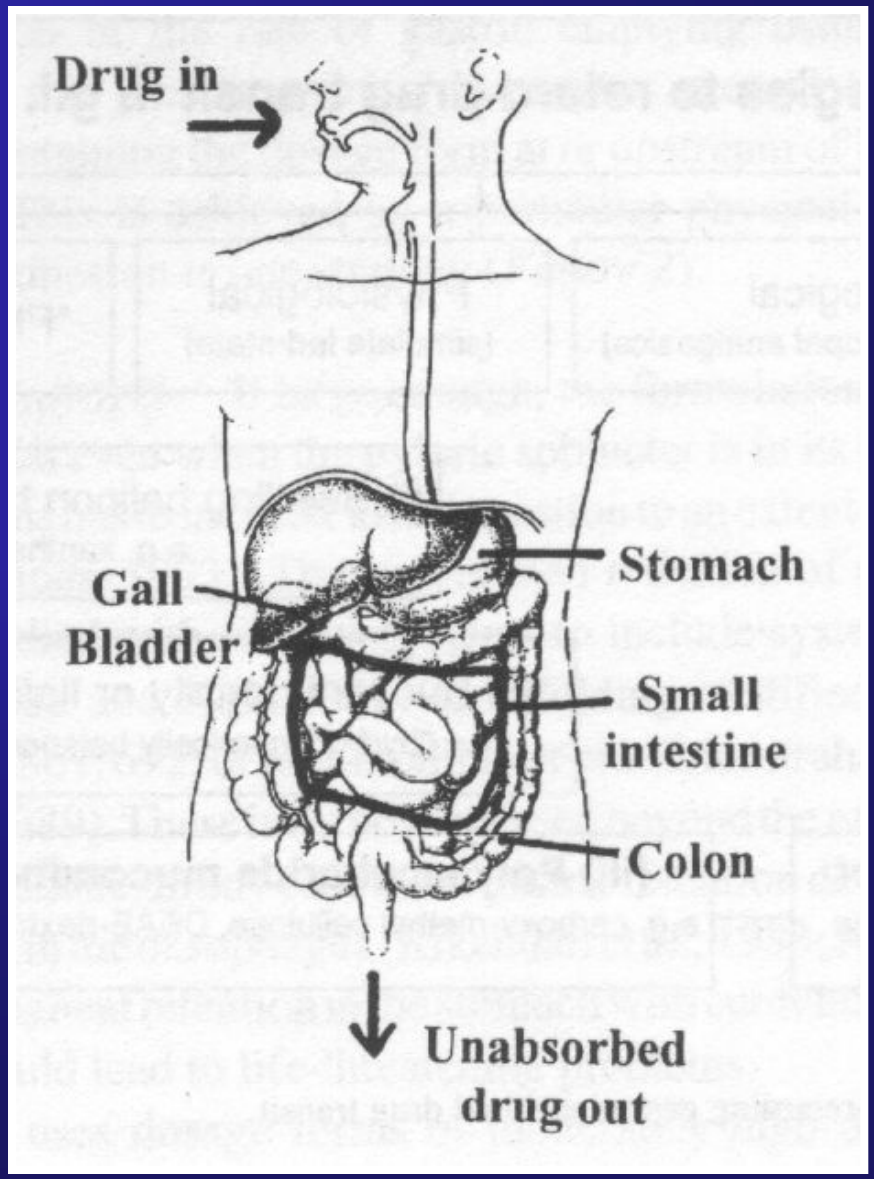


Oral drug administration

Low appearance of drug due to

- too rapid a transit past the ideal absorption site

Macromolecular brakes: MUCOADHESIVES



Now: The mucoadhesive

- non-toxic & not expensive
- high drug loading capacity

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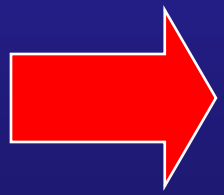


POLYSACCHARIDES

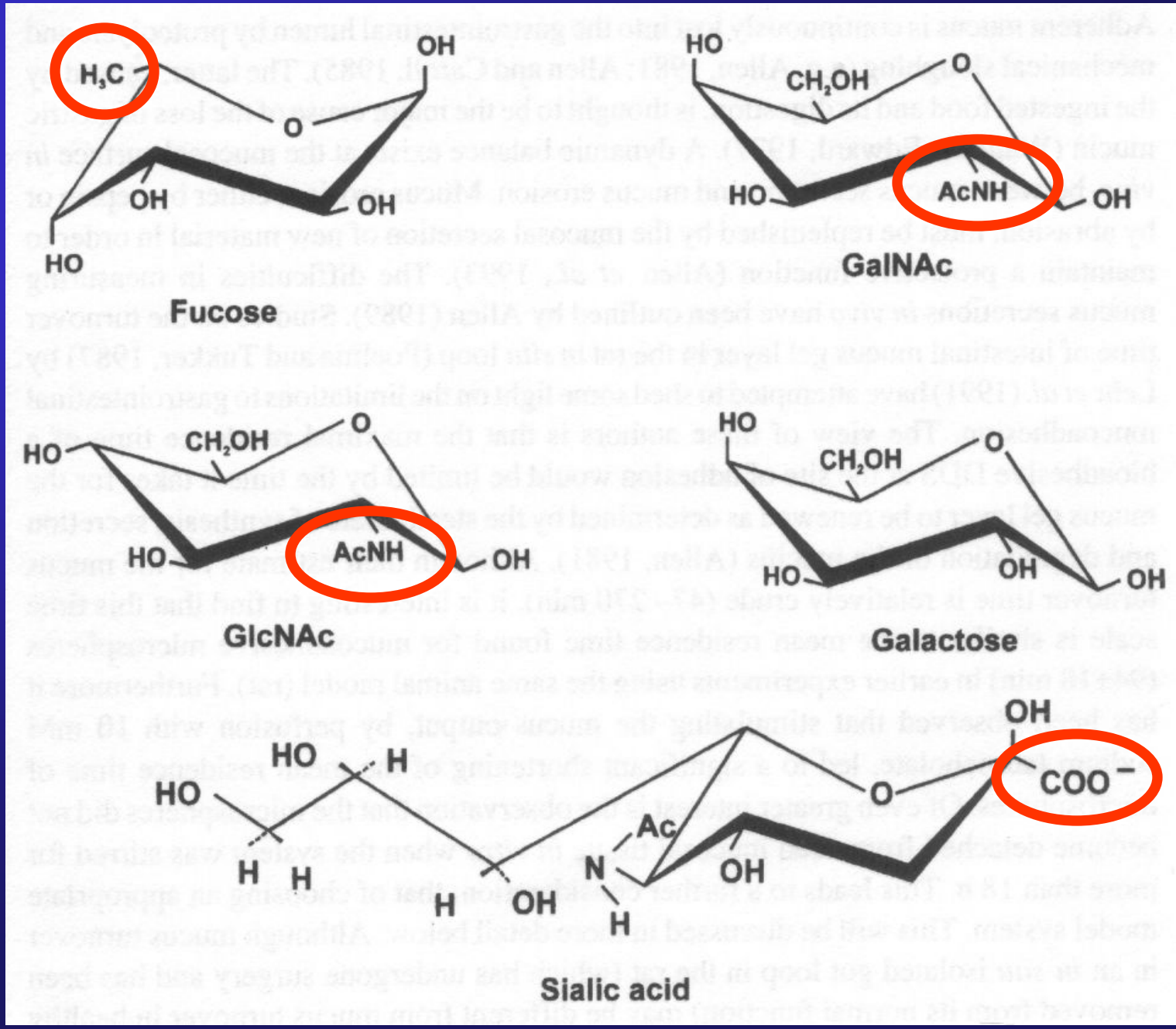

Colgate
Crest
Kolynos
PEPSODENT
CLOSE-UP


Fixodent
Fixodent
DENTURE ADHESIVE POWDER
Extra Hold

Ingredients: Potassium Nitrate (5%), Stannous Fluoride (0.45%), Glycerin And/Or Sorbitol, Water, Hydrated Silica, PEG-40 Castor Oil, PEG-12, Sodium Bicarbonate, Sodium Lauryl Sulfate, Poloxamer 407, Sodium Citrate, Flavor, Titanium Dioxide, Sodium Hydroxide, **Cellulose Gum, Xanthan Gum**, Sodium Saccharin, Stannous Chloride, Citric Acid, Tetrasodium Pyrophosphate, FD&C Blue #1, D&C Yellow #10

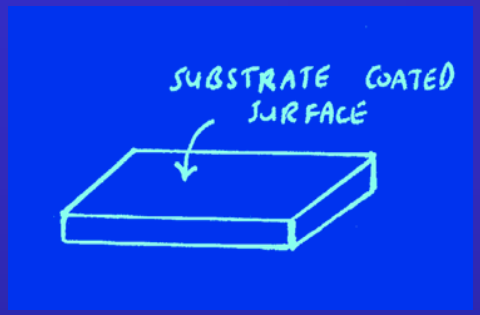
Ingredients:
Polymethyvinylether Maleic Acid Calcium-Zinc Salt, Petrolatum, Mineral Oil, **Cellulose Gum**, Silicon Dioxide, Flavor, Red 27 Aluminum Lake

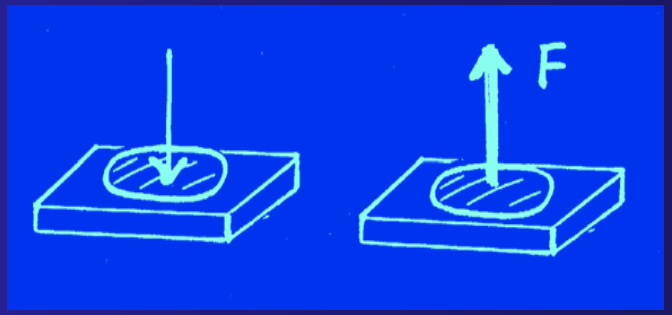
Mucin sugars



Mucoadhesive performance: Tensiometric analysis

{CM Lehr, JA Boustra, EH Schacht, HE Junginger (1992) Int J Pharm.78, 43-48}





Neutral polysaccs	***F*** **(mN/cm^2)**
HP-cellulose	~0 (2.8±2.8)
HE-starch	~0 (0.6±0.8)
Scleroglucan	~0
Anionic polysaccs	
Pectin	~0
Xanthan	~0
CMC (low visc)	1.8±1.1
CMC (medium)	0.3±0.3
CMC (high visc)	1.3±1.0

Chitosans	***F*** **(mN/cm^2)**
Wella low-visc.	3.9±1.2
Wella high-visc	6.7±0.7
Knapezyk	5.7±1.1
Daichitosan-H	8.0+5.7
Daichitosan-VH	9.5±2.4
Sea-Cure 240	4.1±2.9
Sea-Cure 210+	9.5+2.5
Sigma	6.6±3.0
Cationic dextrans	
DEAE-dextran	~0
Amino-dextran	~0

Molecular assay methods

- Viscometry/ rheology
- Surface plasmon resonance
- Dynamic light scattering
- Turbidity/ light scattering
- **SEC-MALLS**/ FFF-MALLS
- **Analytical ultracentrifuge**
- **Electron microscopy**
- **Atomic Force Microscopy**

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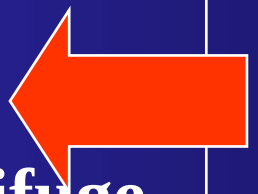
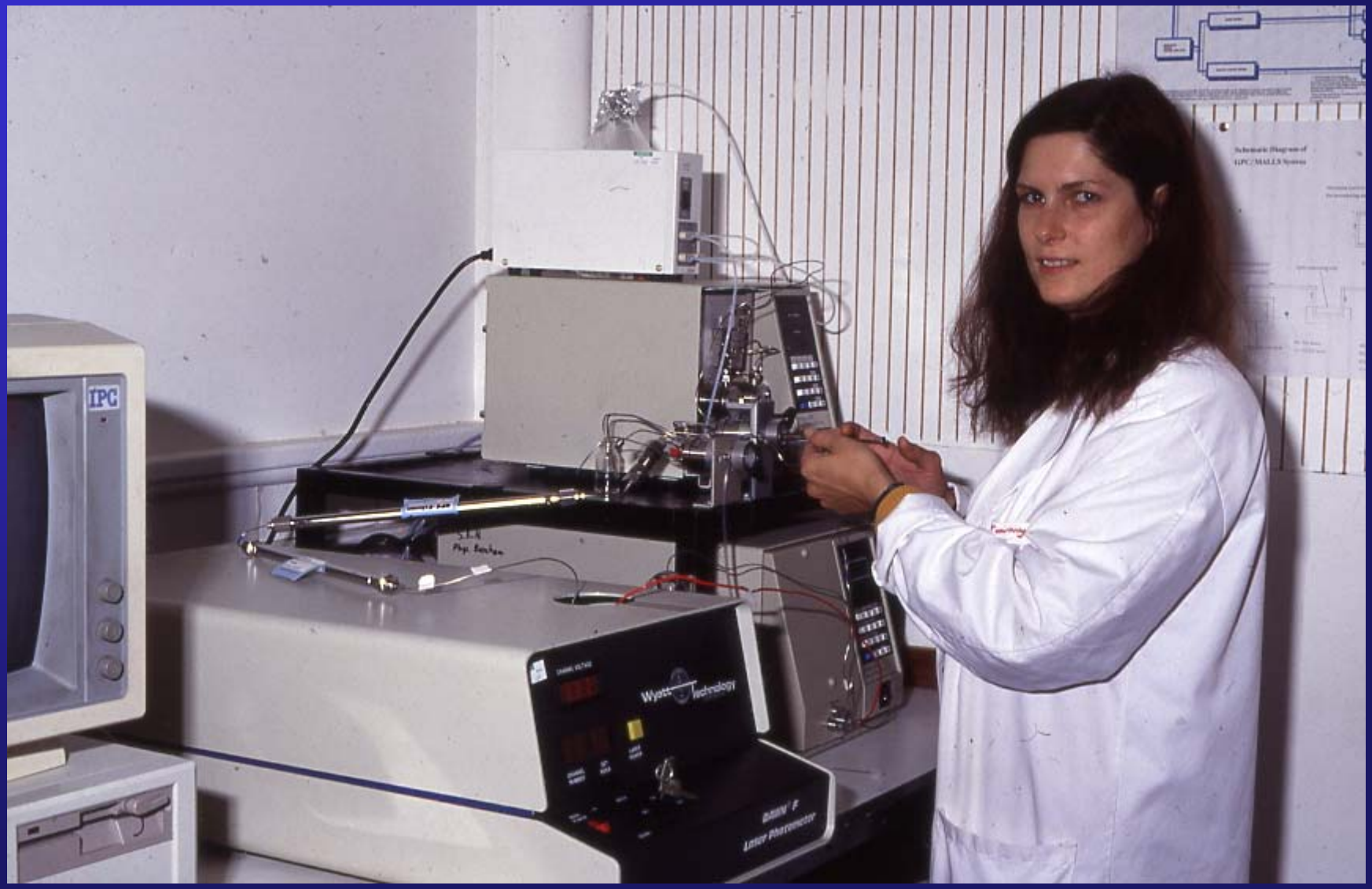
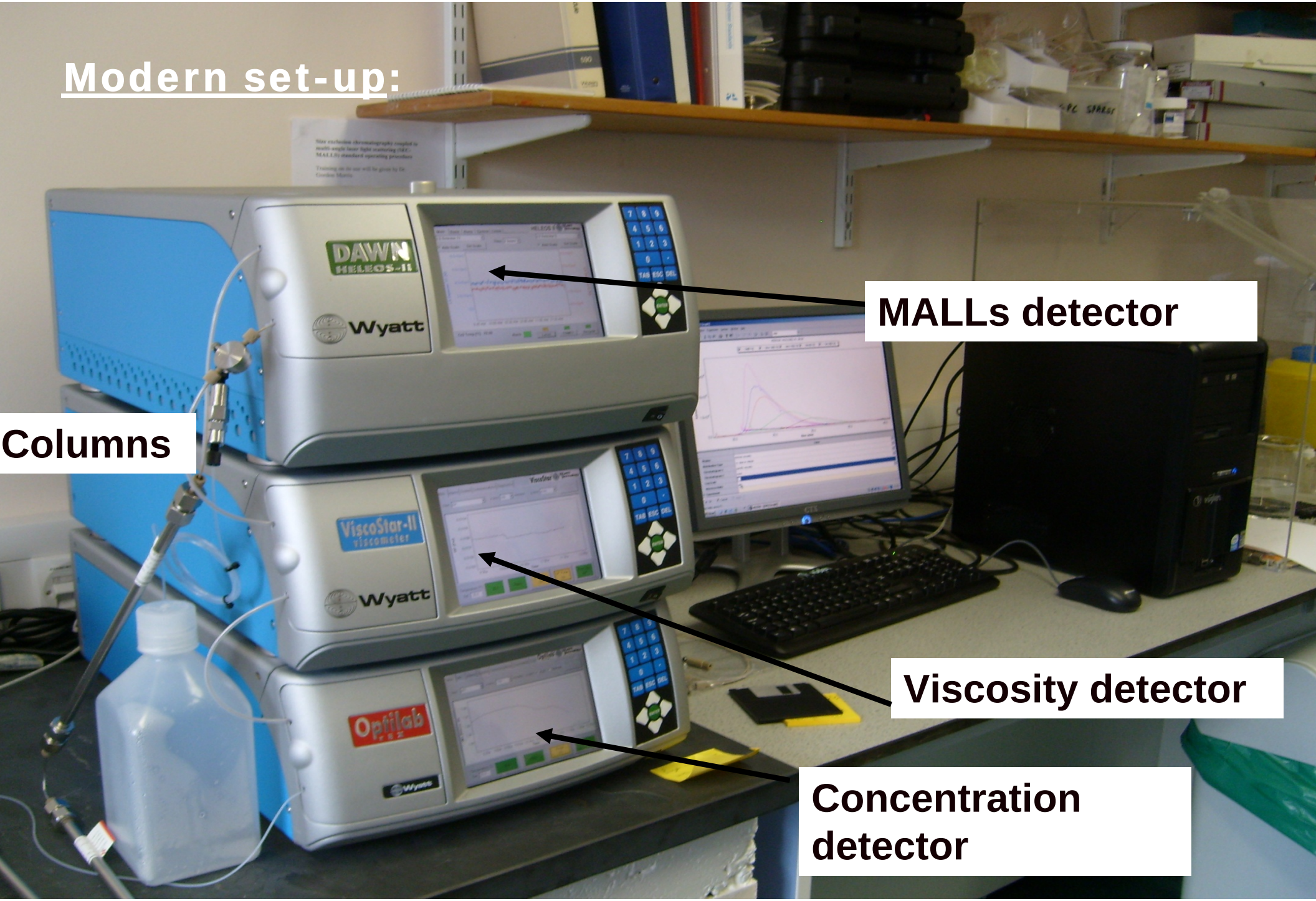


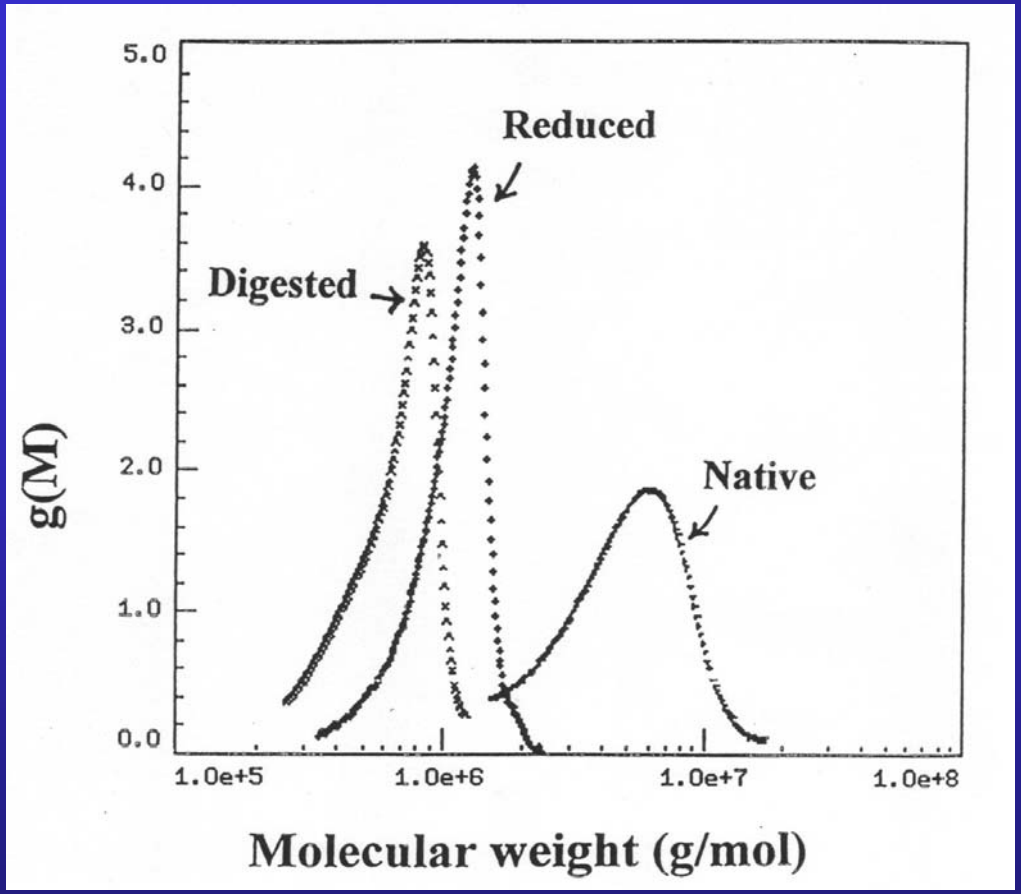


Dr. Conny Jumel

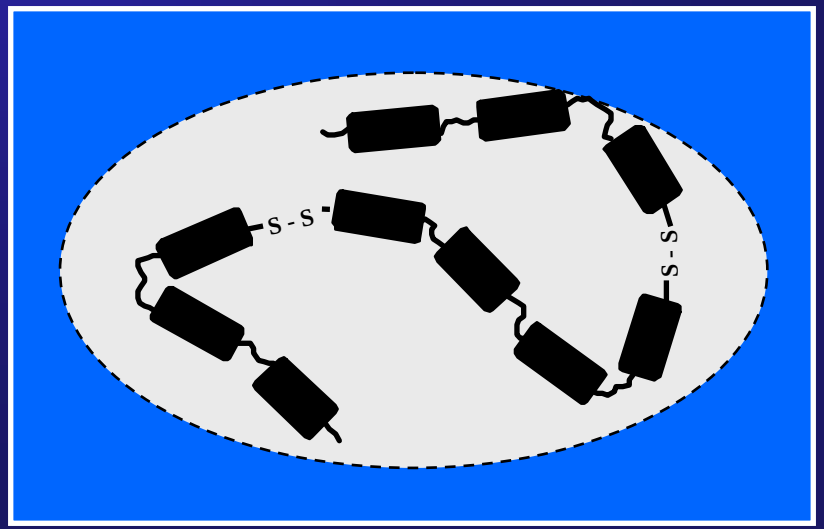
Modern set-up:



Molecular weight distribution for colonic mucin



Linear Random Coil structure



Jumel K, Fogg F, Allen A, Harding SE, 1997

Molecular assay methods

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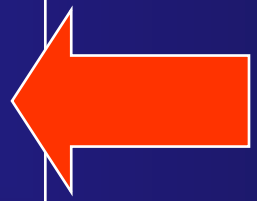
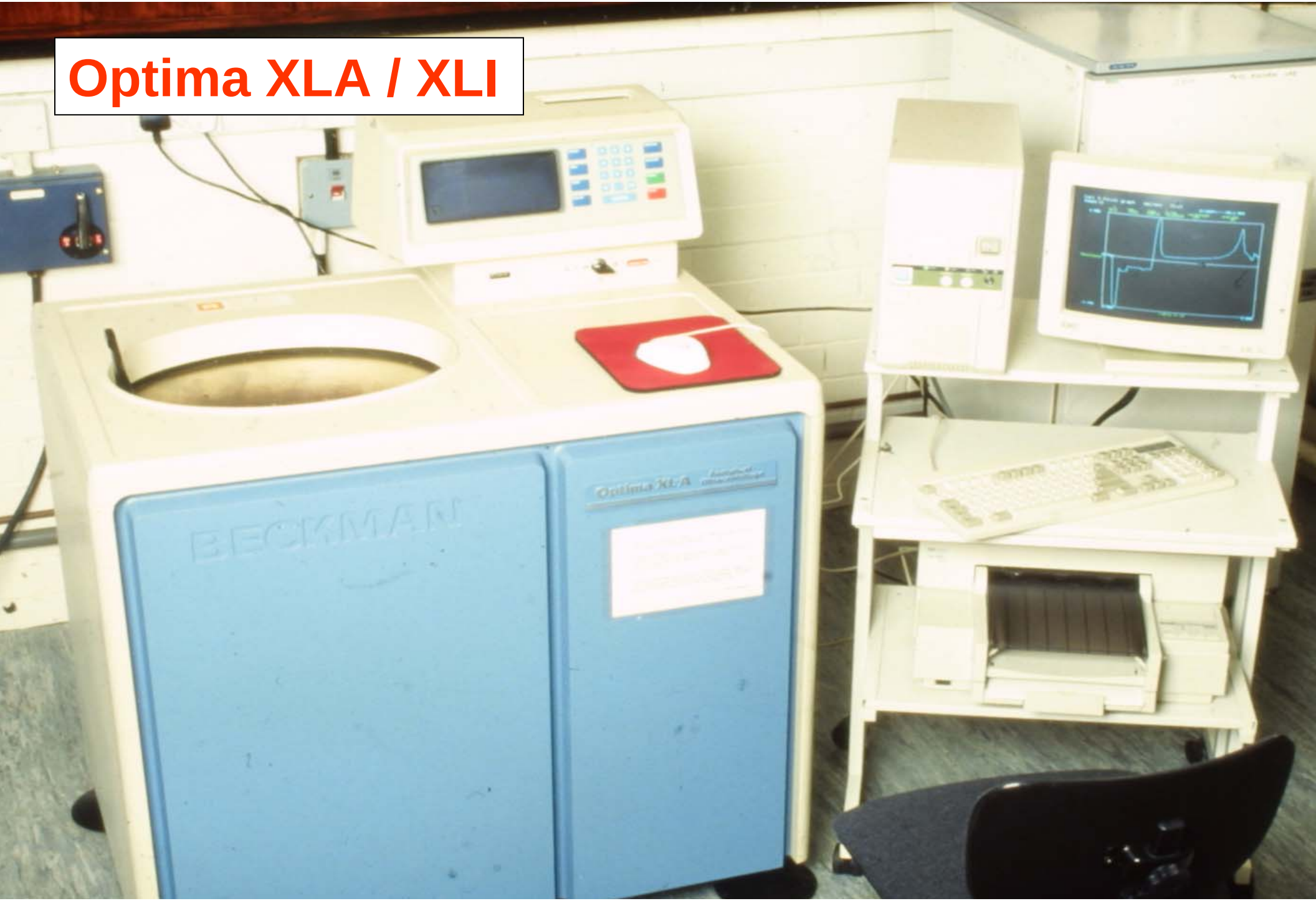
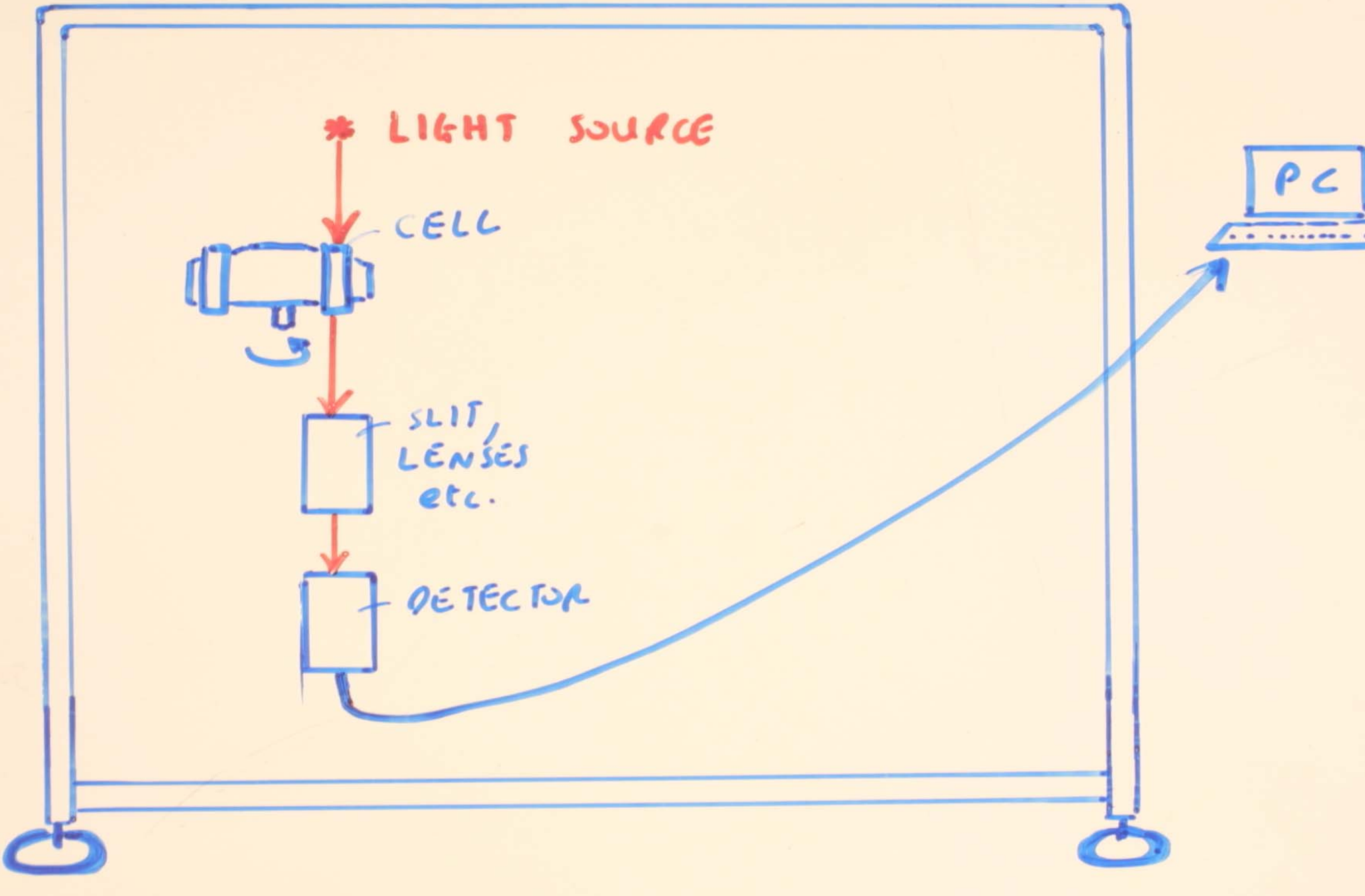
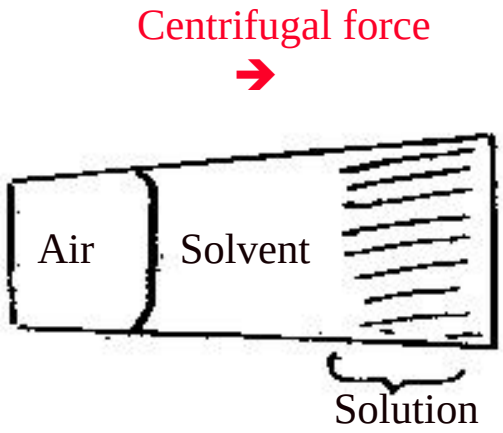


Optima XLA / XLI

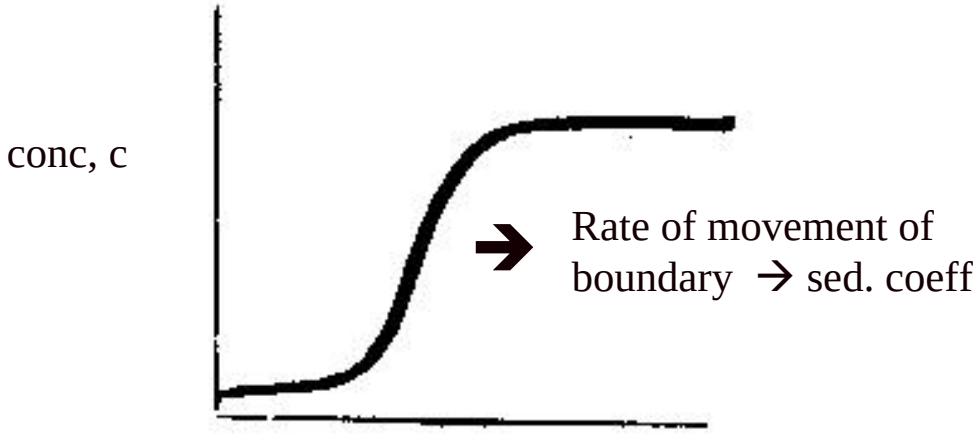



LIGHT SOURCE
CELL
SLIT, LENSES etc.
DETECTOR
PC

Sedimentation Velocity in the Analytical Ultracentrifuge



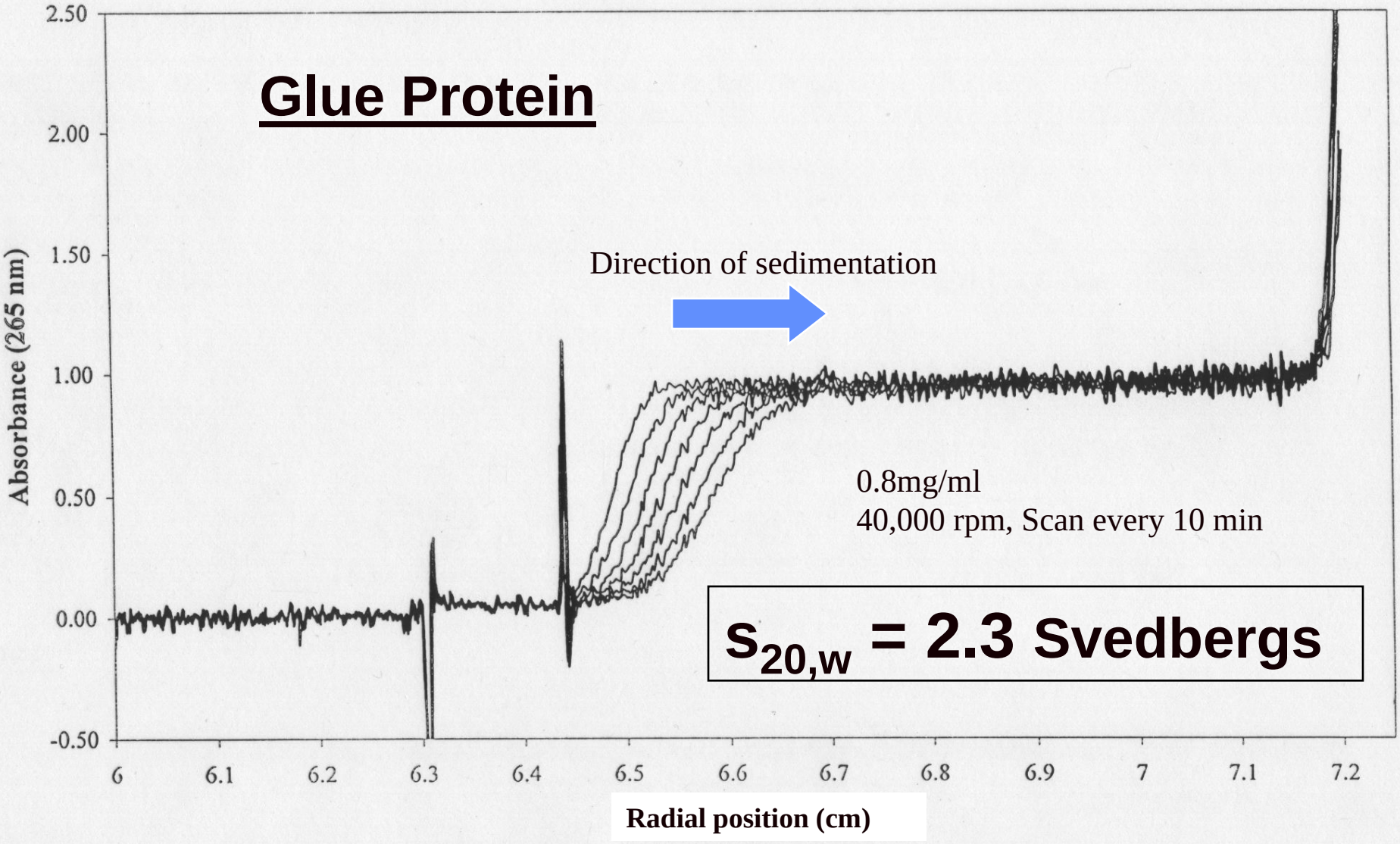
Top view, sector of centrifuge cell



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

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

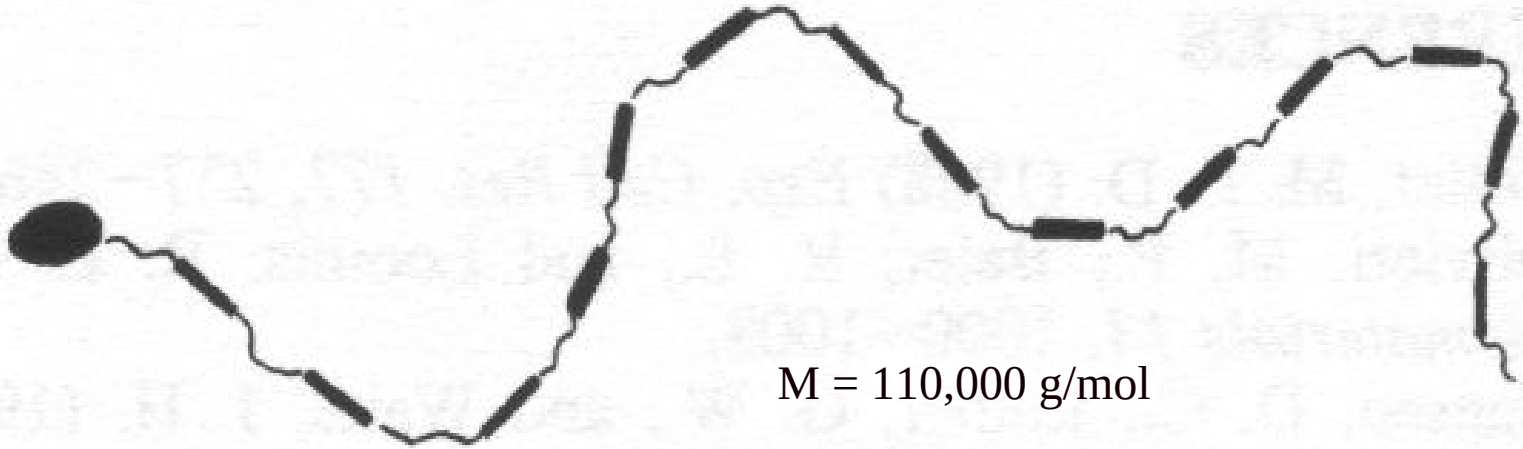
Glue Protein



$s_{20,w}$ = 2.3 Svedbergs


Mussels ➔ “Glue foot protein” *mefp1*

Mussel Glue Protein Hydrodynamics



M = 110,000 g/mol

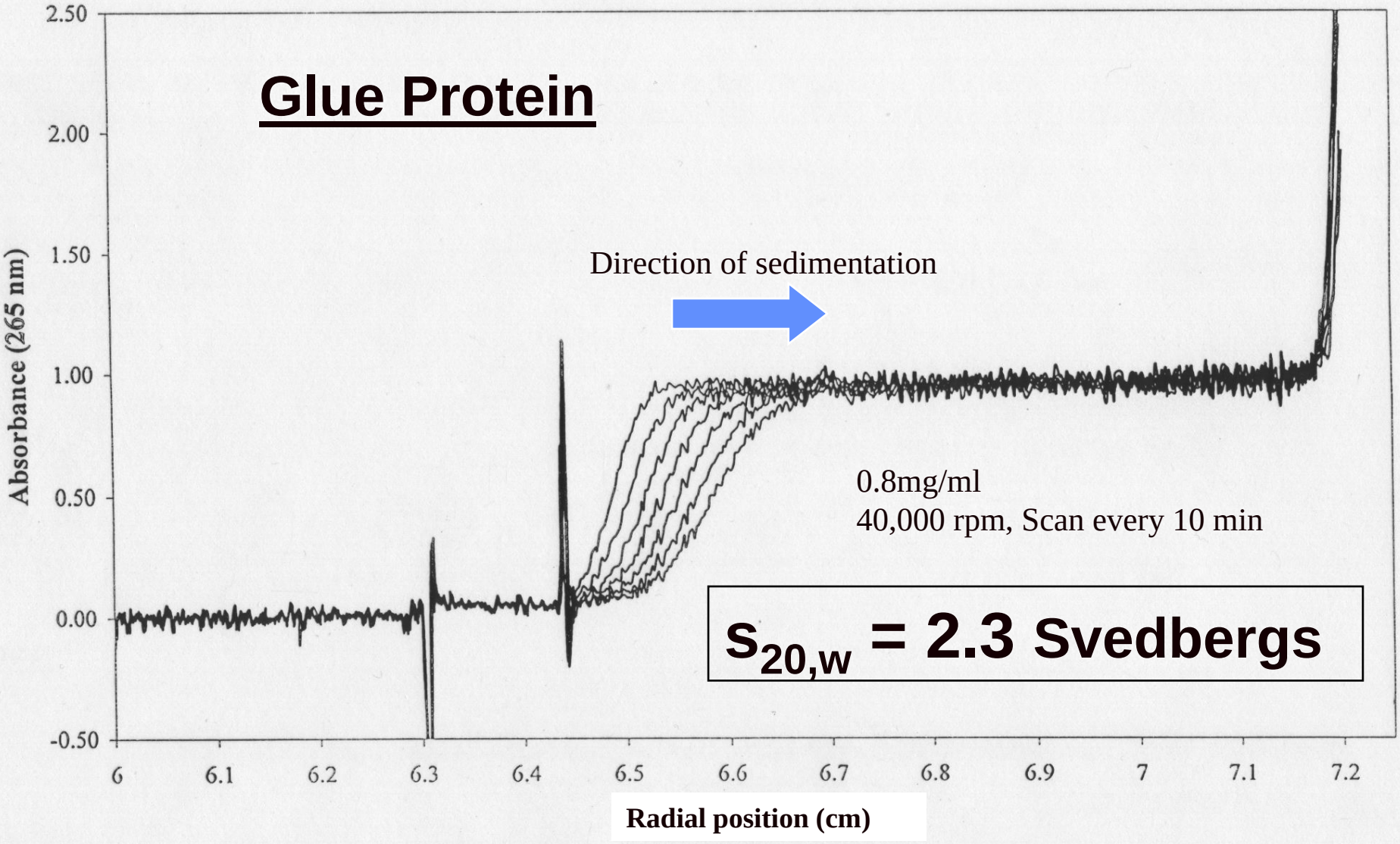
: non-repetitive globular region

: flexible segment [P*P*TYK]

: rigid segment [AKPSY]

Deacon, Waite, Davis & Harding, Biochemistry, 1998

Glue Protein



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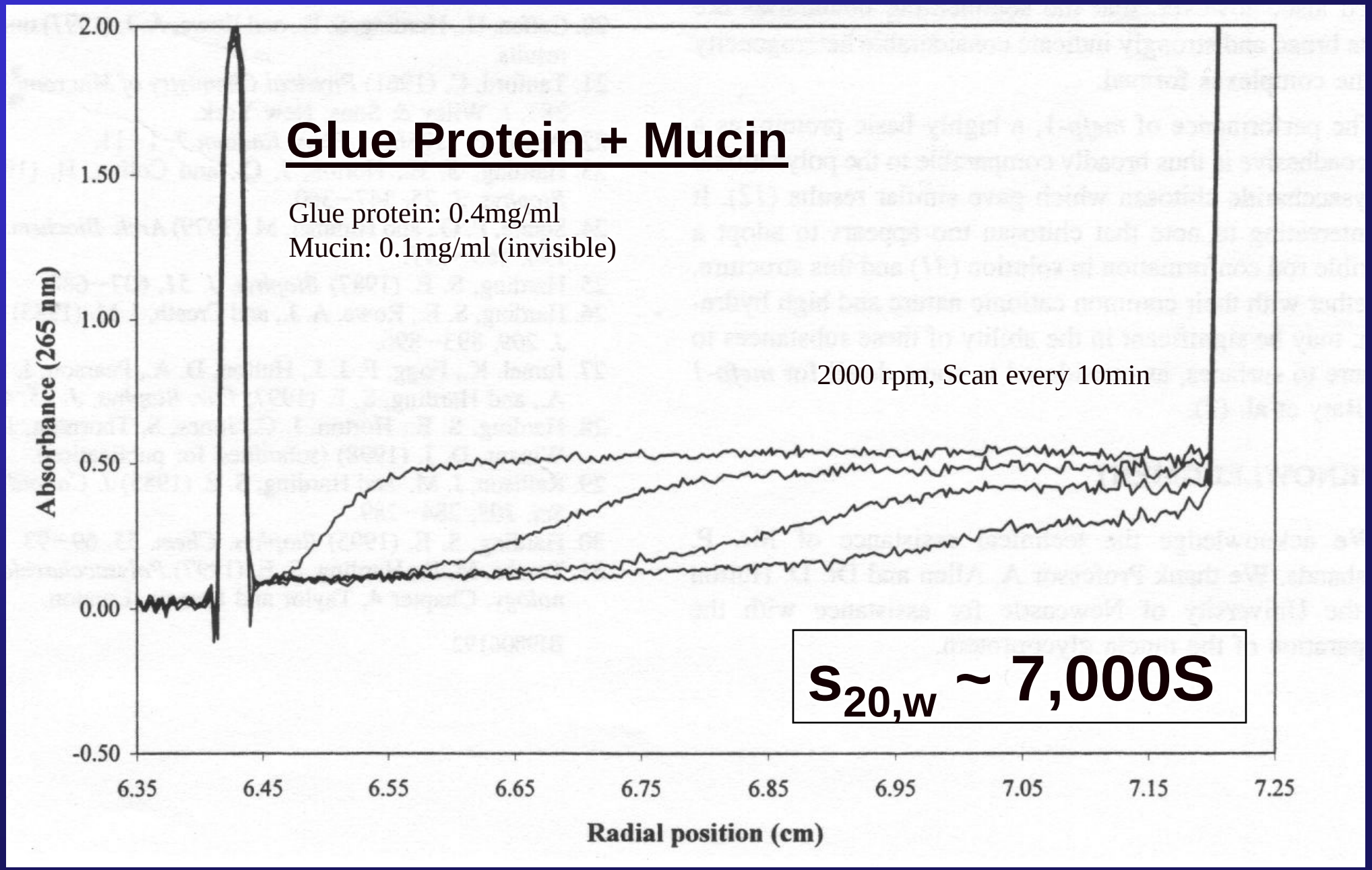
Glue Protein + Mucin
Glue protein: 0.4mg/ml
Mucin: 0.1mg/ml (invisible)
2000 rpm, Scan every 10min
$s_{20,w}$ ~ 7,000S
Absorbance (265 nm)
2.00
1.50
1.00
0.50
0.00
-0.50
6.35
6.45
6.55
6.65
6.75
6.85
6.95
7.05
7.15
7.25
Radial position (cm)

Candidate Polysaccharides:

Guar

Alginate

Carboxy-methyl cellulose

Xanthan

DEAE-dextran

Chitosans

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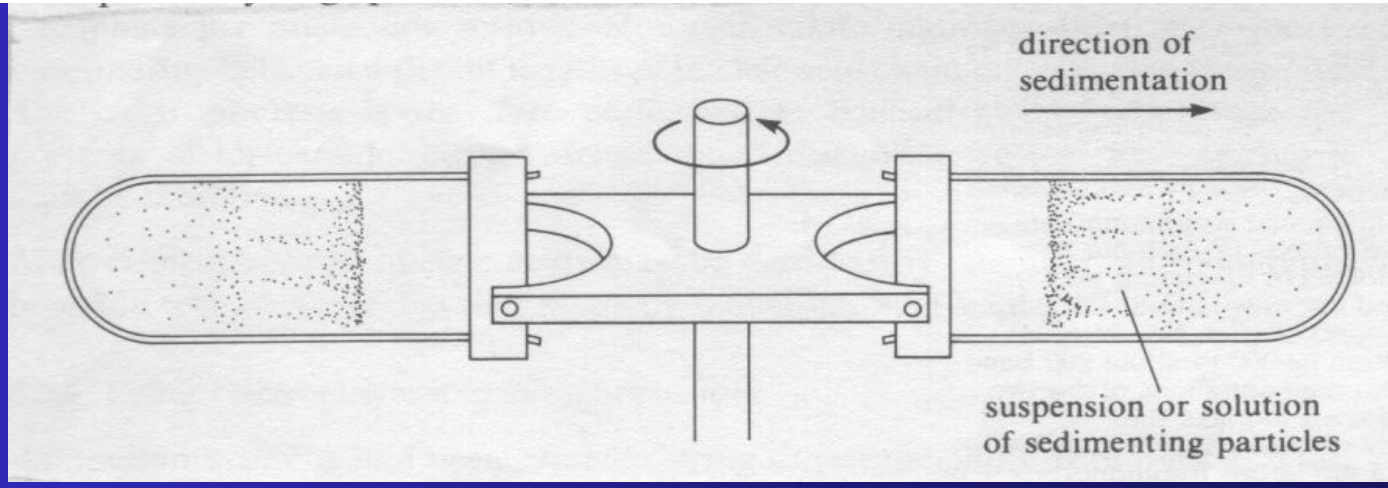
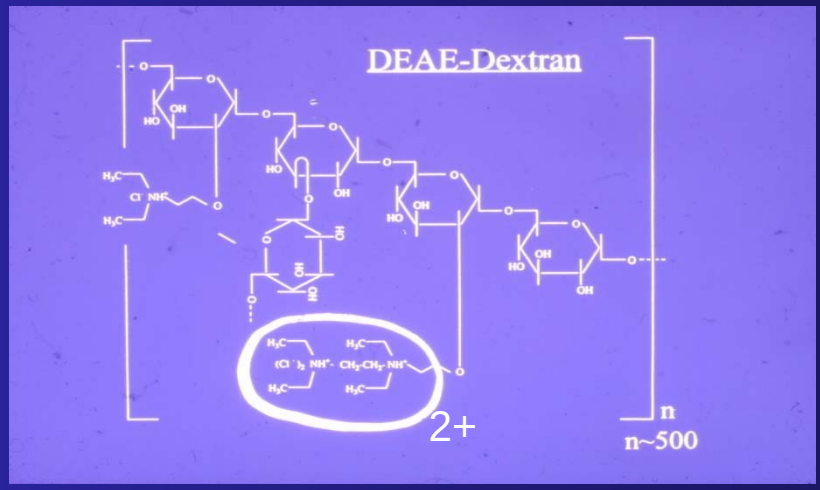


Simple criterion for an interaction:

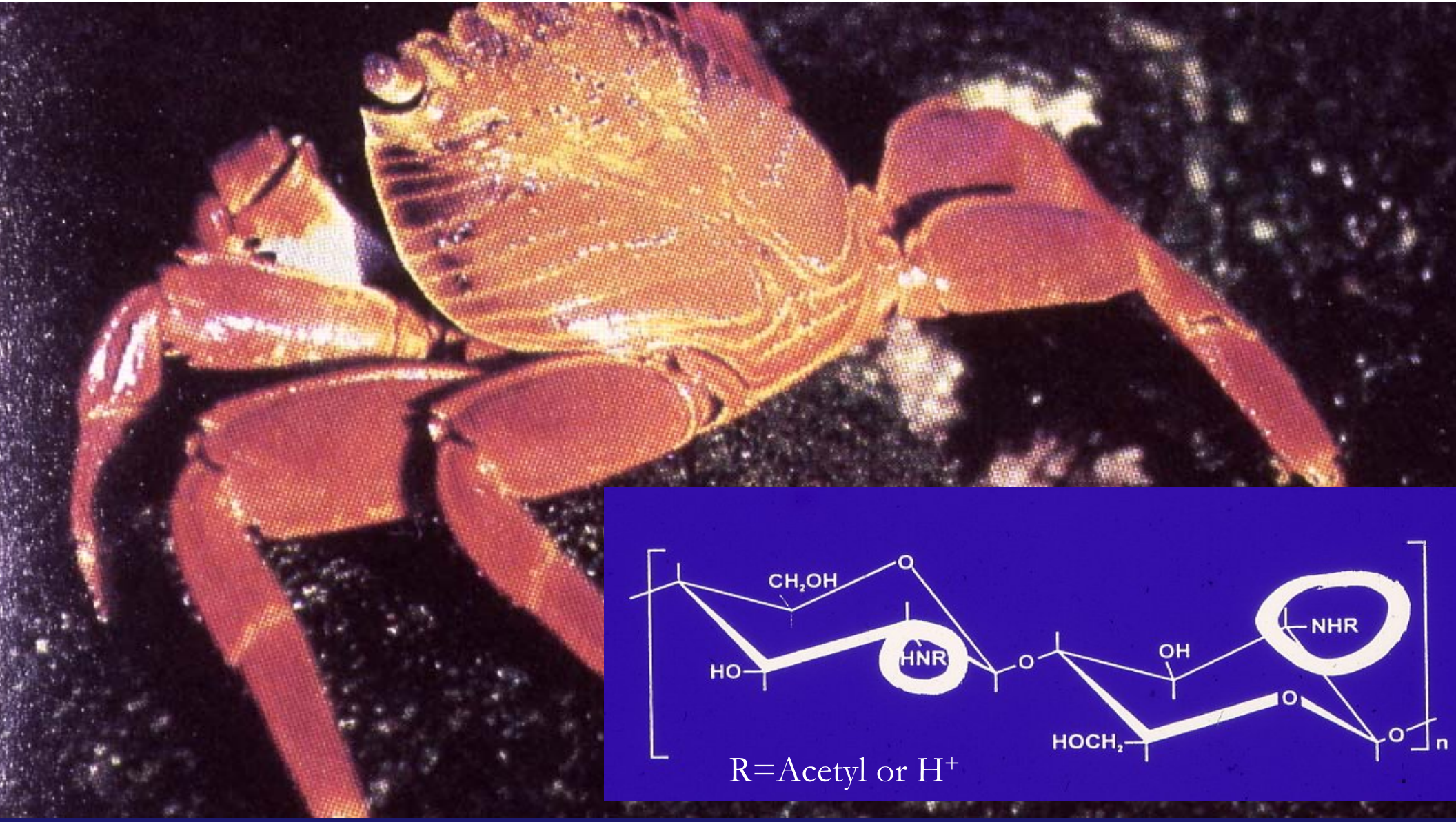
RATIO:

$$\frac{\text{Sedimentation value of complex}}{\text{Sedimentation value of mucin}}$$

DEAE dextran



mucin:DEAE-dextran ratio	Buffer+temp	s_{mucin} (S) control	s_{mix} (S) complex	s_{mix}/s_{mucin}
2.0: 1.9 (mg/ml)	pH6.8, I=0.1, 20 °C	17	19	1.1
	" " 37 °C	17	20	1.2
1.8: 3.2	" " "	18	25	1.4
0.2: 1.0	" " 20 °C	35	65	1.9
	pH7.0 tris " "	42	55	1.3



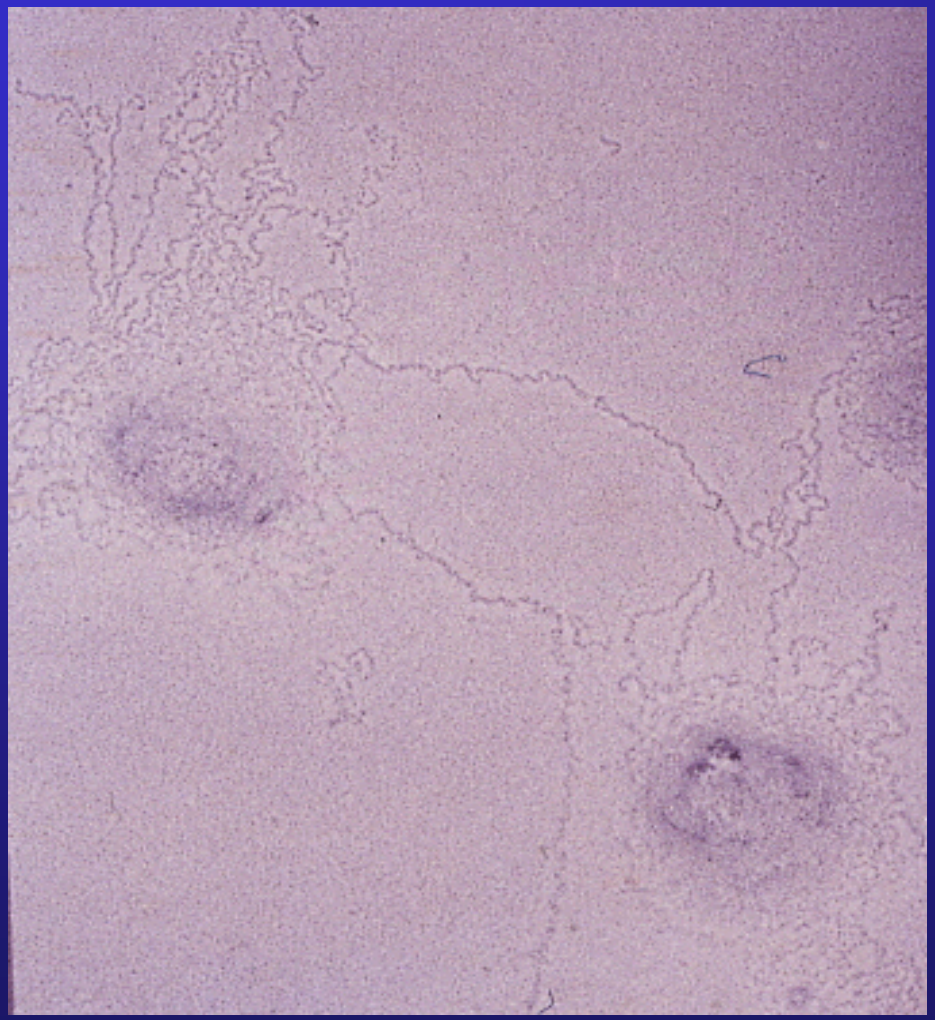
Crabshell: ⇨ chitin ⇨ chitosan

Sedimentation velocity assay: mucin+*chitosans*

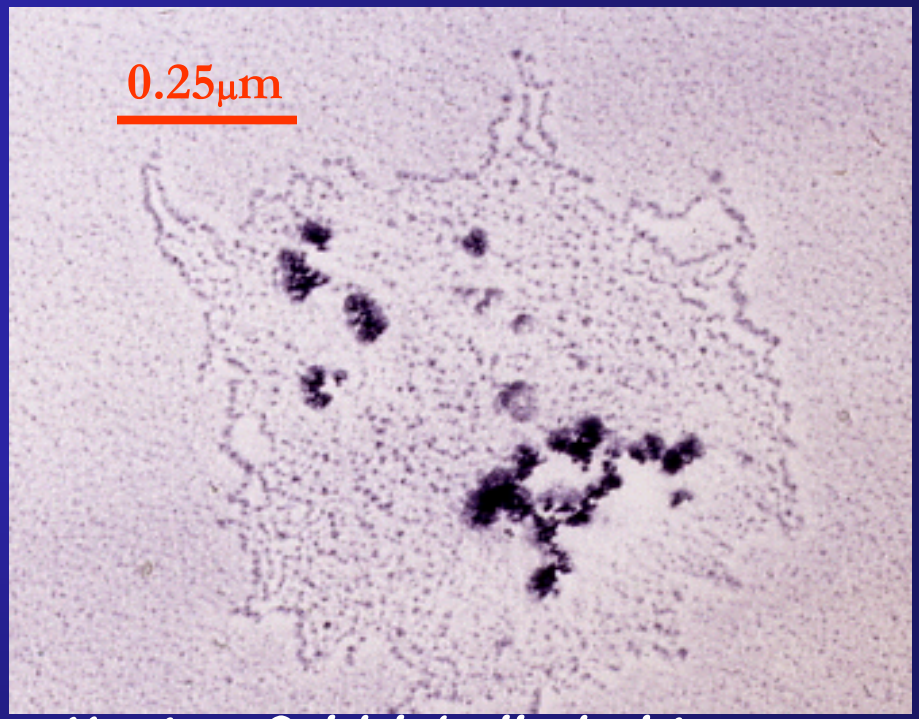
UV absorption optics (Beckman XL-A ultracentrifuge)
chitosan controls: sed. coeff. *s* ~ 1.5 Svedbergs (S)
mucin: chitosan ratio 0.2mg/ml : 1.0mg/ml
Sea-Cure +210 (Pro-Nova, Drammen): degree of acetylation F_A ~ 0.11
KN50 (NTH-Trondheim) " " " ~ 0.42

chitosan	Buffer+temp	s_{mucin} (S) **control**	s_{mix} (S) **complex**	s_{mix}/s_{mucin}
sea-cure +210	pH4.5, I=0.1, 20°C	53	780	15
	" " 37°C	53	1990	38
KN50 Trondheim	pH4.5, I=0.1, 20°C	53	1630	31
	" " 37°C	53	2340	44

Images of mucin/chitosan complexes using Electron Microscopy



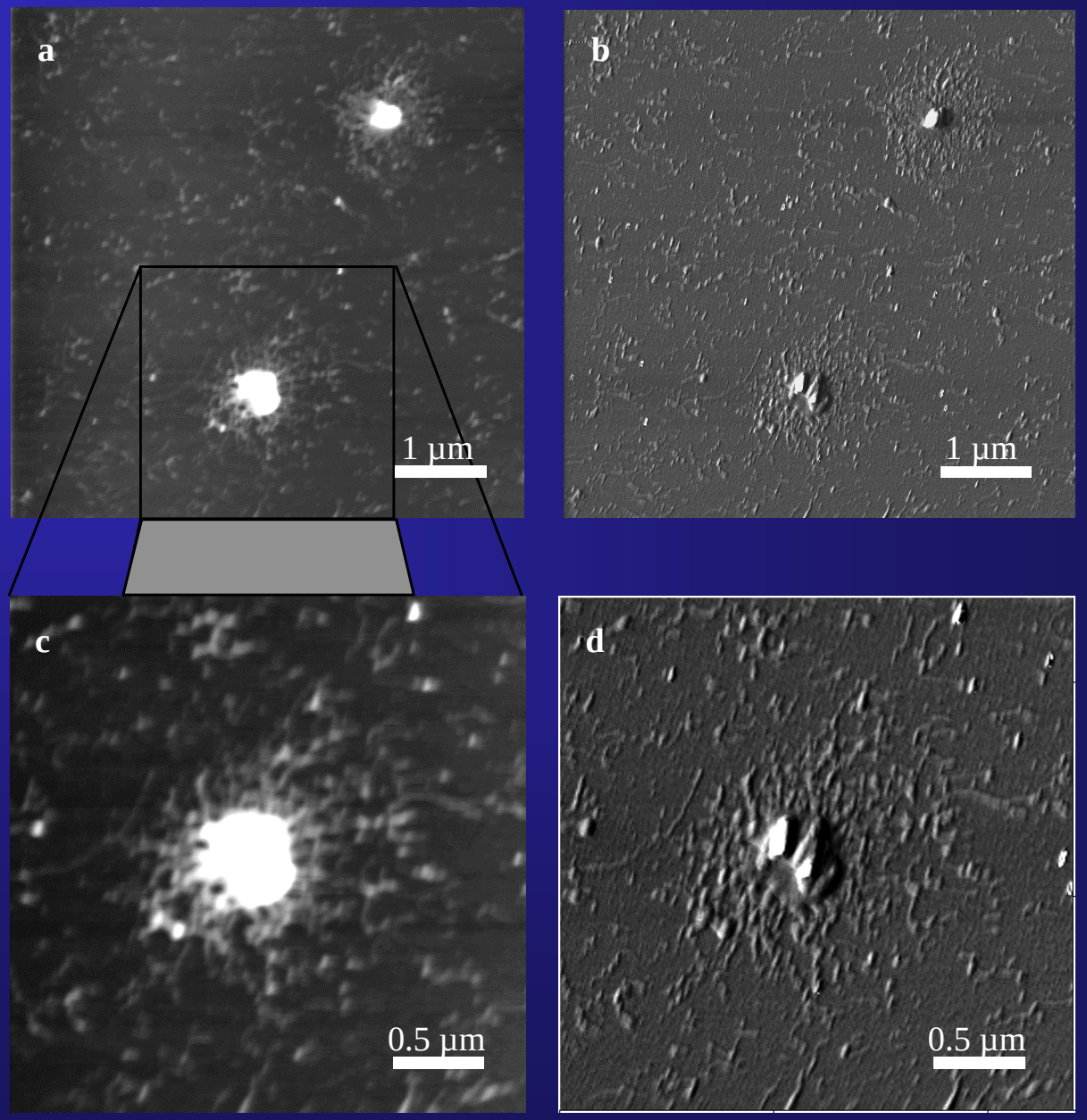
Mucin-chitosan



Mucin- Gold labelled chitosan

Fiebrig et al, 1996

Images of mucin/chitosan complexes using Atomic Force Microscopy

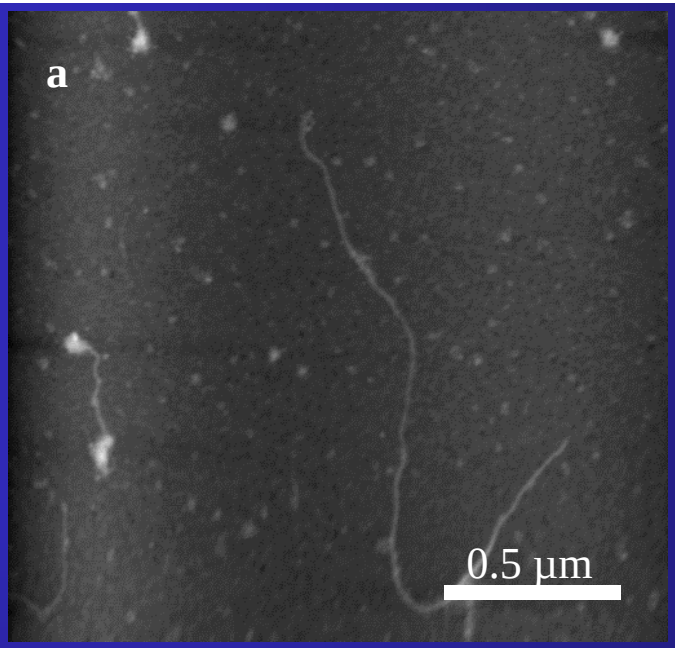


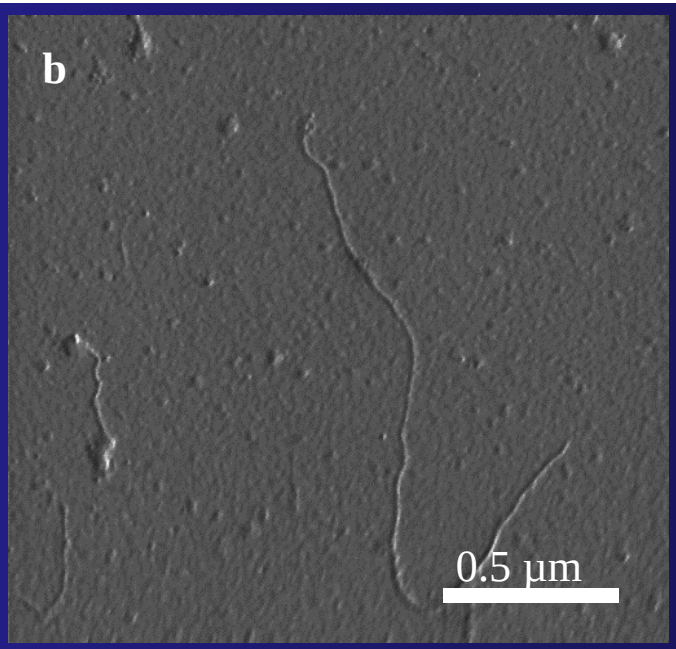
Deacon, McGurk, Roberts, Williams, Tendler, Davies, Davis & Harding (2000), *Biochem. J.* 348, 557

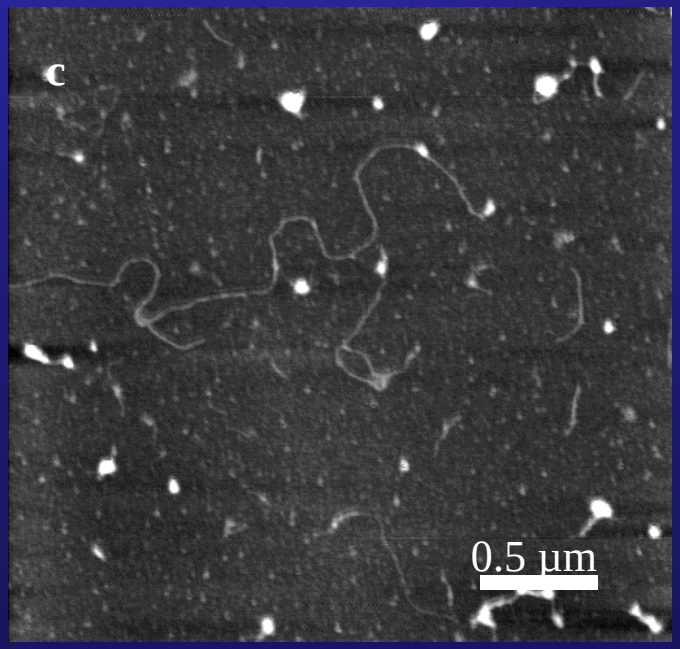
Atomic force microscopy: mucin

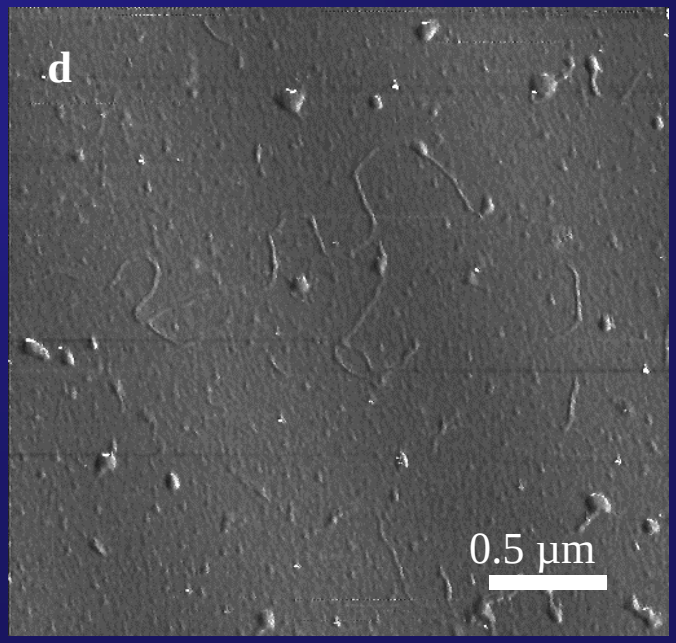
a&c: topography mode

b&d: phase mode







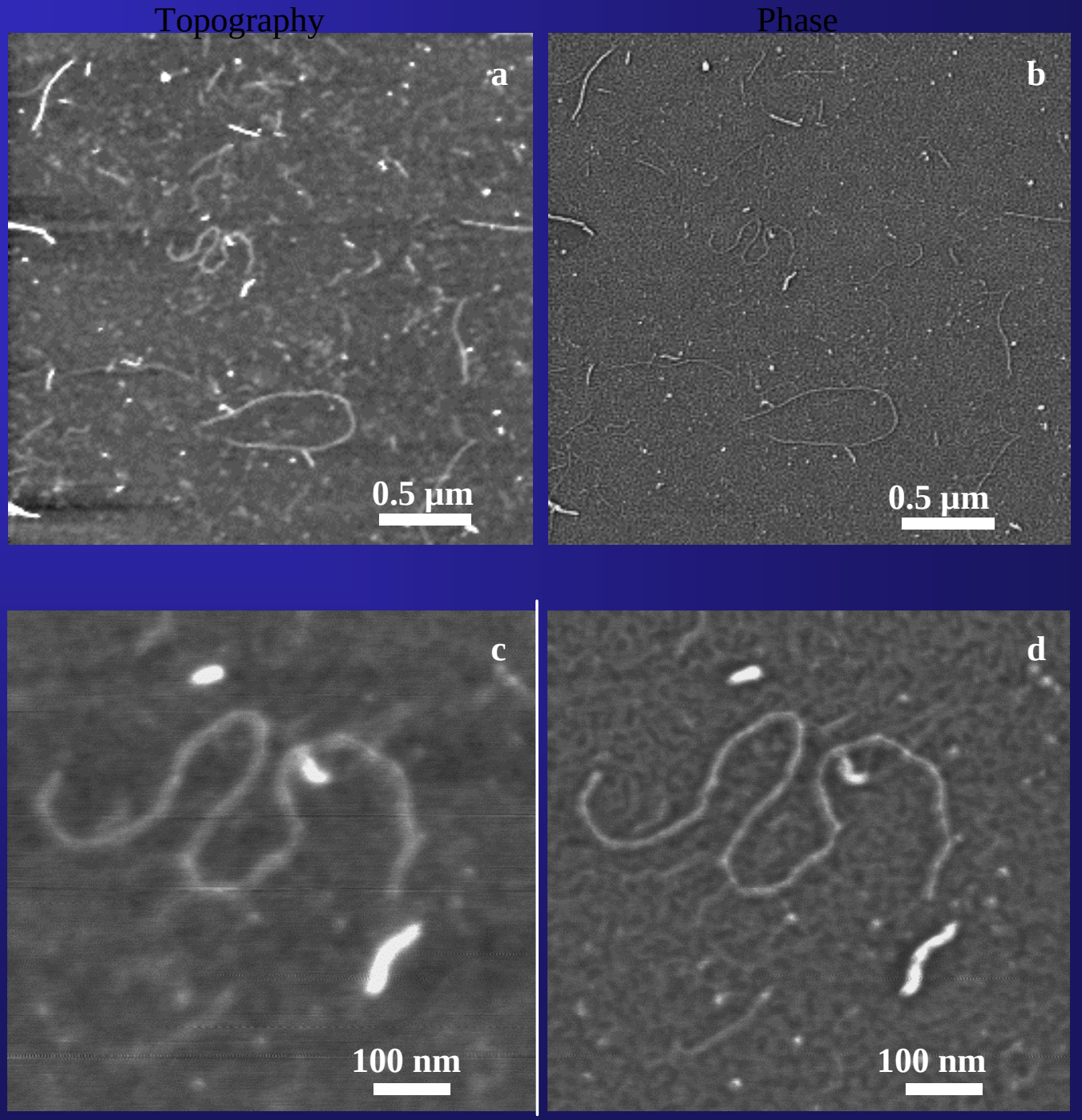


Deacon, McGurk, Roberts, Williams, Tendler, Davies, Davis & Harding (2000), *Biochem. J.* 348, 557

Atomic force microscopy: chitosan

a&c: topography mode

b&d: phase mode



Deacon, McGurk, Roberts, Williams, Tendler, Davies, Davis & Harding (2000), *Biochem. J.* 348, 557

Sedimentation velocity assay: mucin+*chitosan (sea cure 210+)* ***Effect of pH***

pH	**Temp**	s_{mucin} **(S) control**	s_{mix} **(S) complex**	s_{mix}/s_{mucin}
2.0	20°C	45	980	22
	37°C	132	1626	12
4.5	20°C	53	780	15
	37°C	53	1990	38
6.5	20°C	32	1524	48
	37°C	46	1580	34

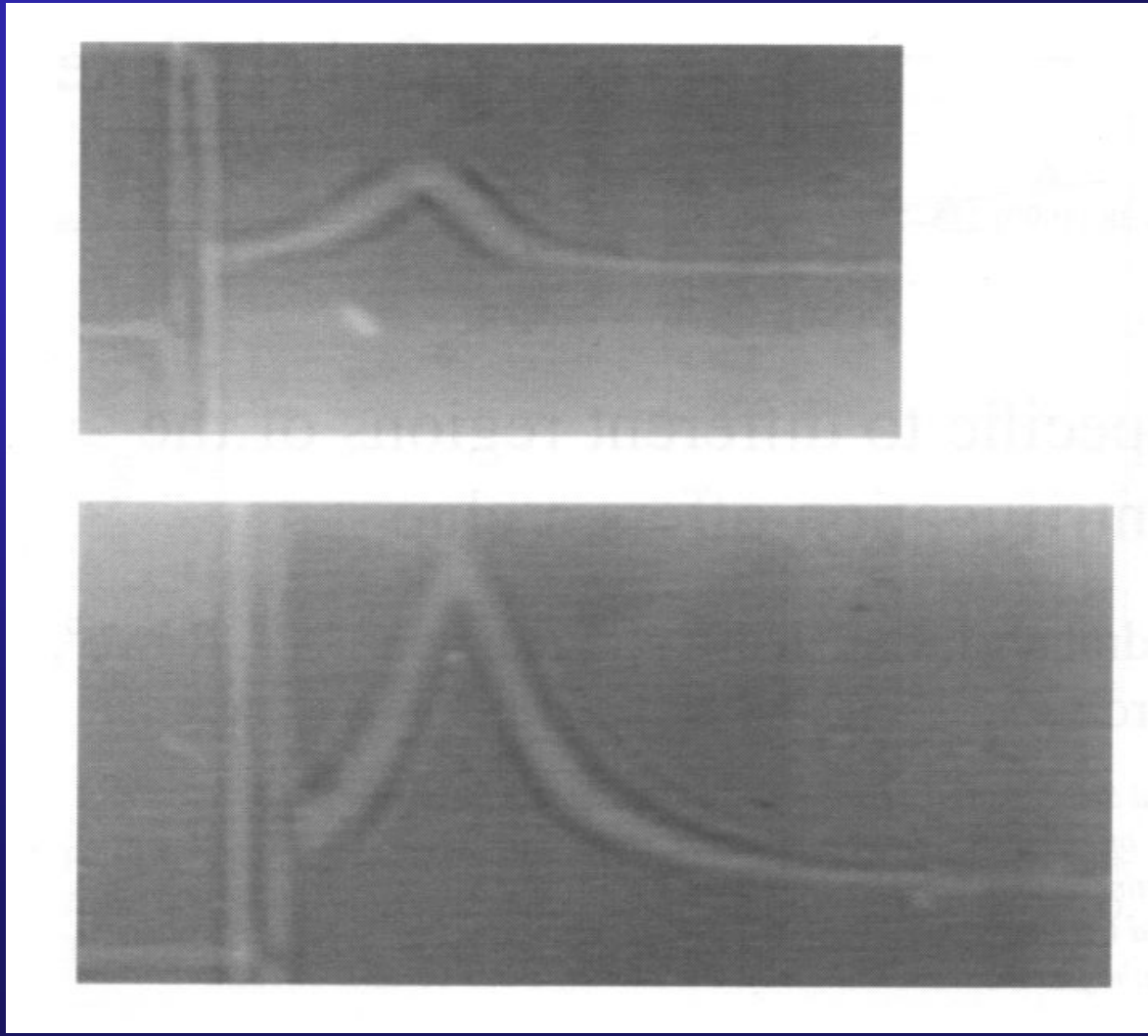
Sedimentation Fingerprinting:

assay for mucoadhesive interaction where mucin concentration is too small to be detected

Schlieren Optical system:

Chitosan-mucin mixture:

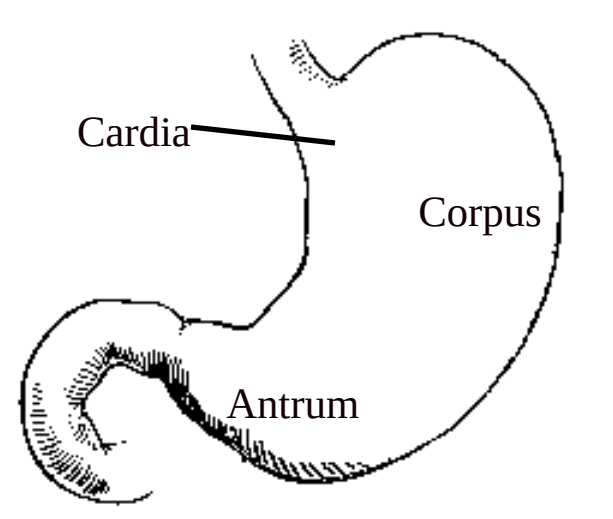
Chitosan control:

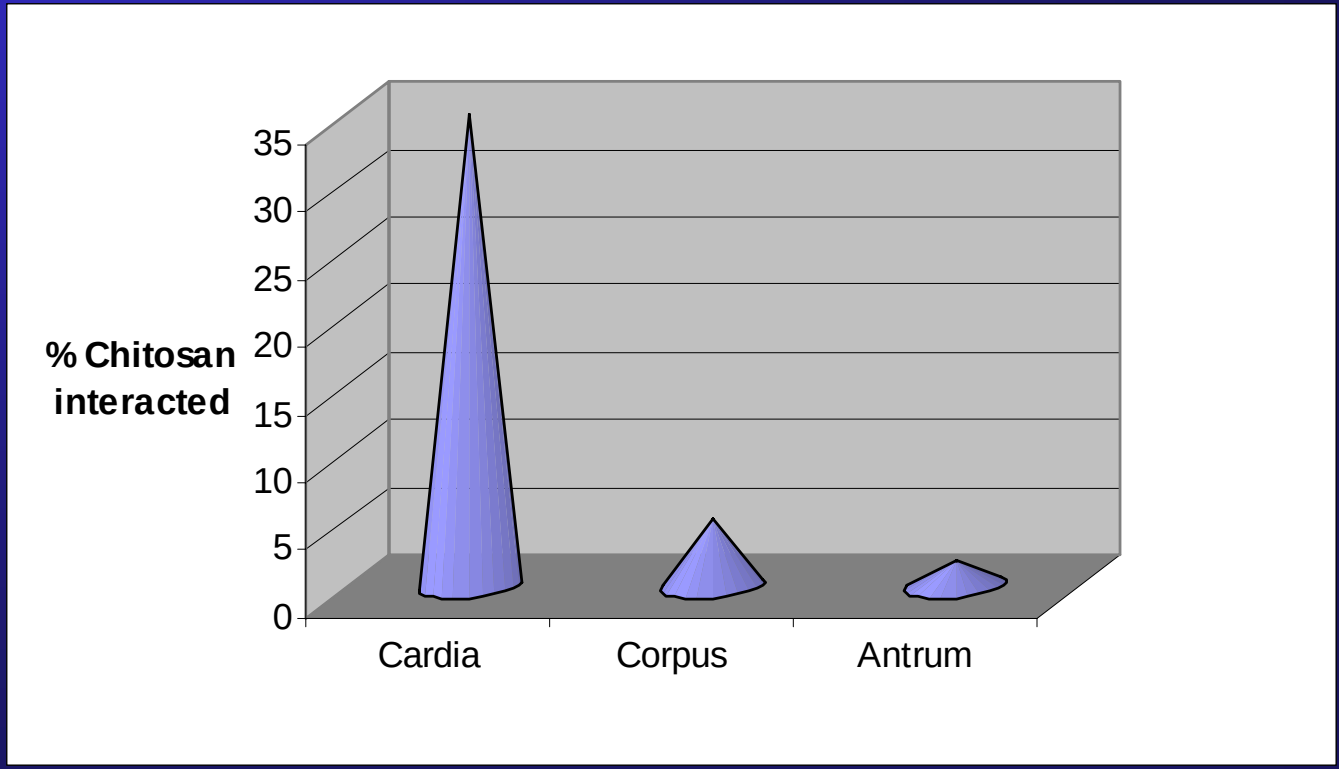


Loss of concentration of chitosan through complex formation as index for interaction

Chitosan-mucin interactions specific to different regions of the stomach

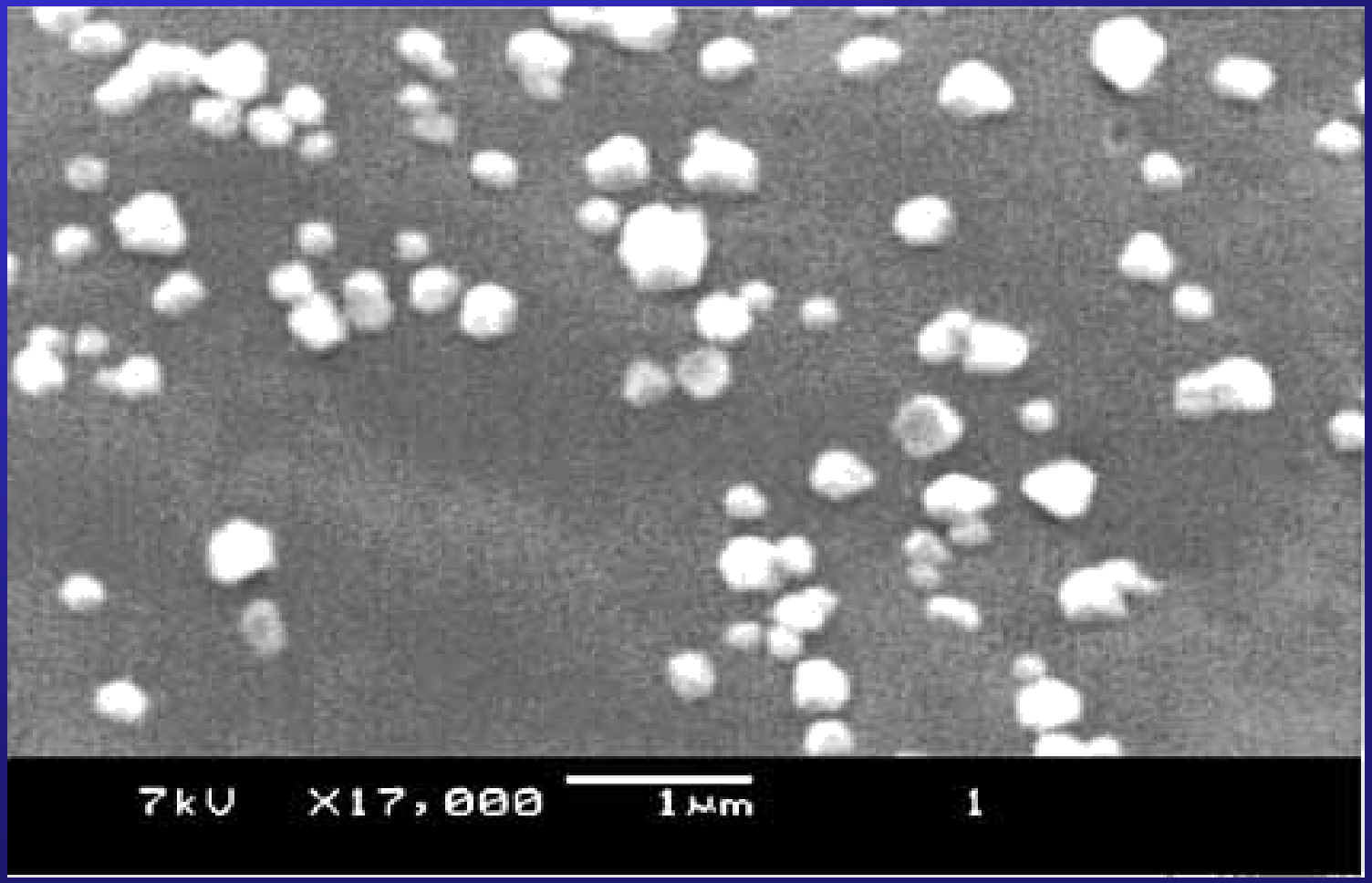
Deacon, Davis, White, Nordman, Carlstedt, Errington, Rowe & Harding, 1999





Chitosan: F_A = 0.11, Initial concentrations: chitosan 4 mg/ml, mucin <1 mg/ml, I=0.1M

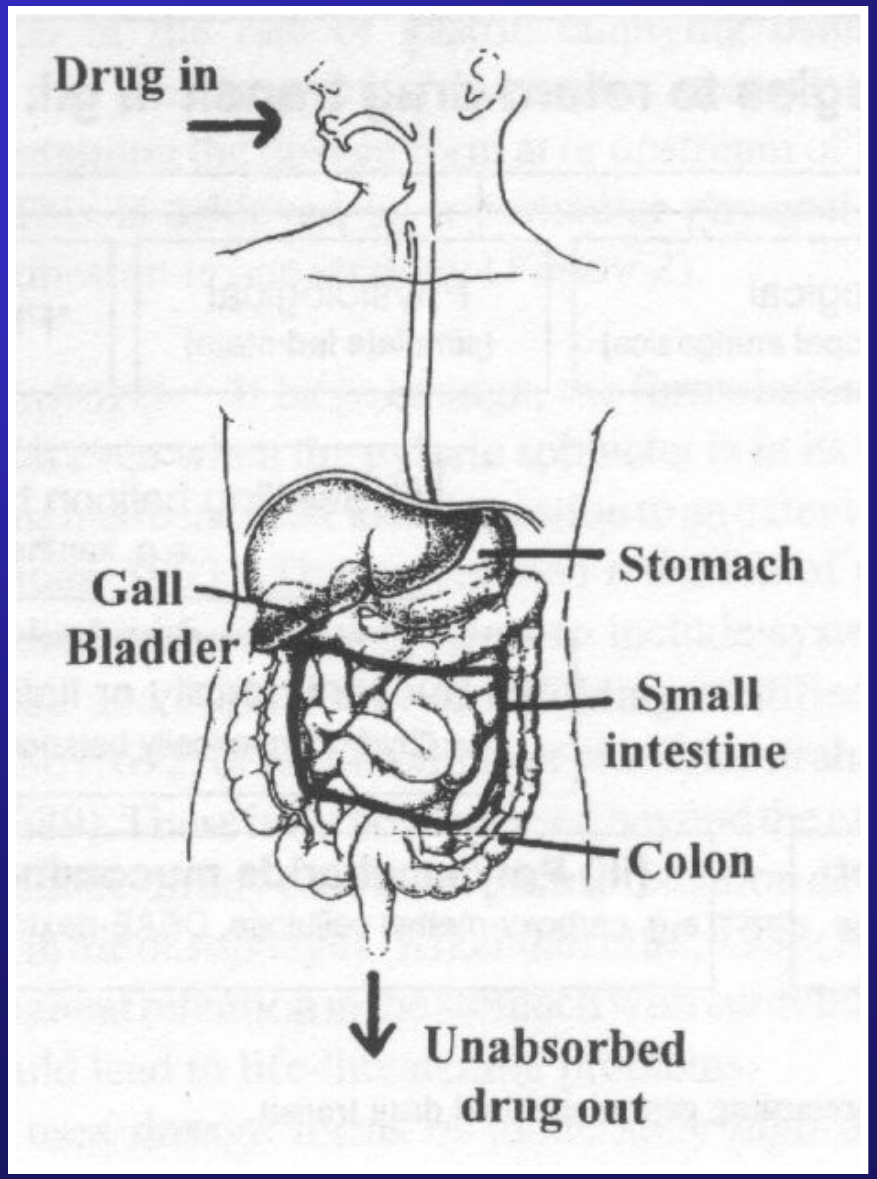
Oral formulations need nanoparticles/ microparticles



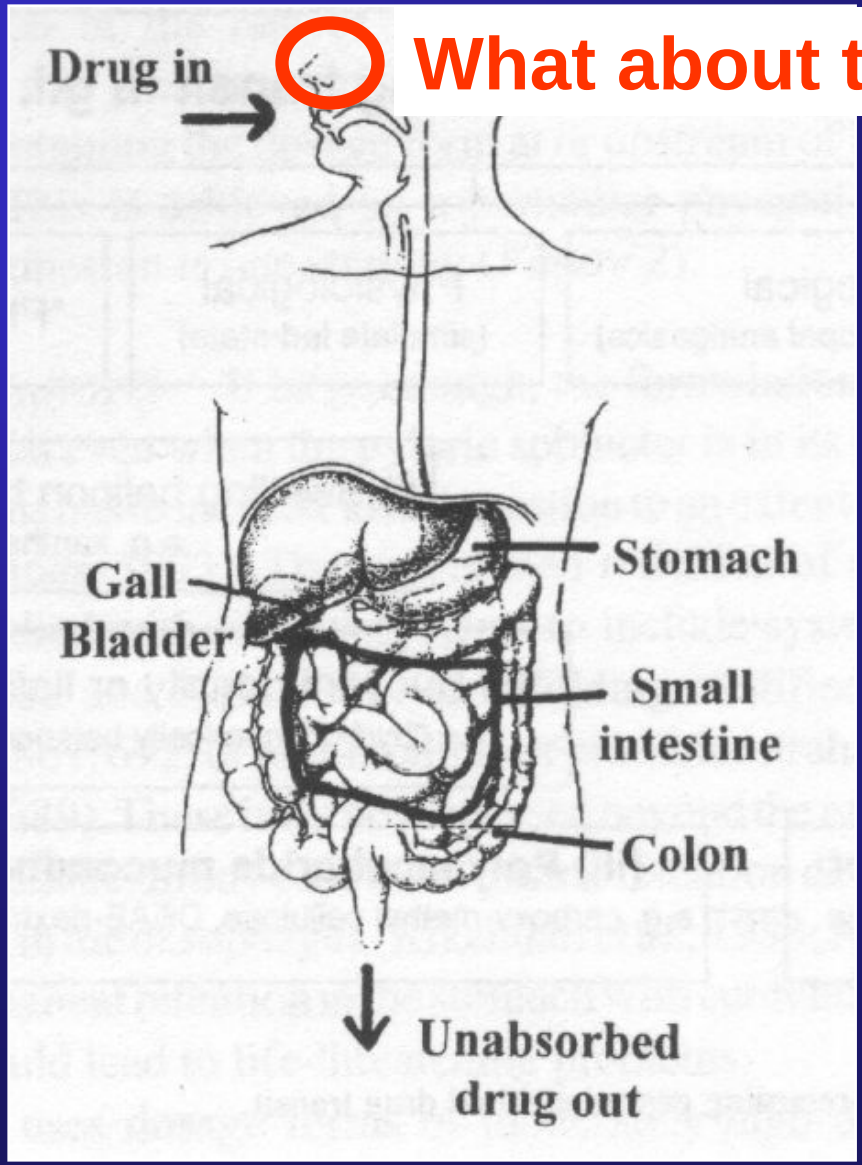
Prepared with tripolyphosphate at pH 5.3 with insulin loading concentration of 4.28 μg/ml.

Zengshaun *et al*., (2002), *Journal of Pharmaceutical Sciences*, 91(6) 1396-1404

Oral drug administration?



Oral drug administration?

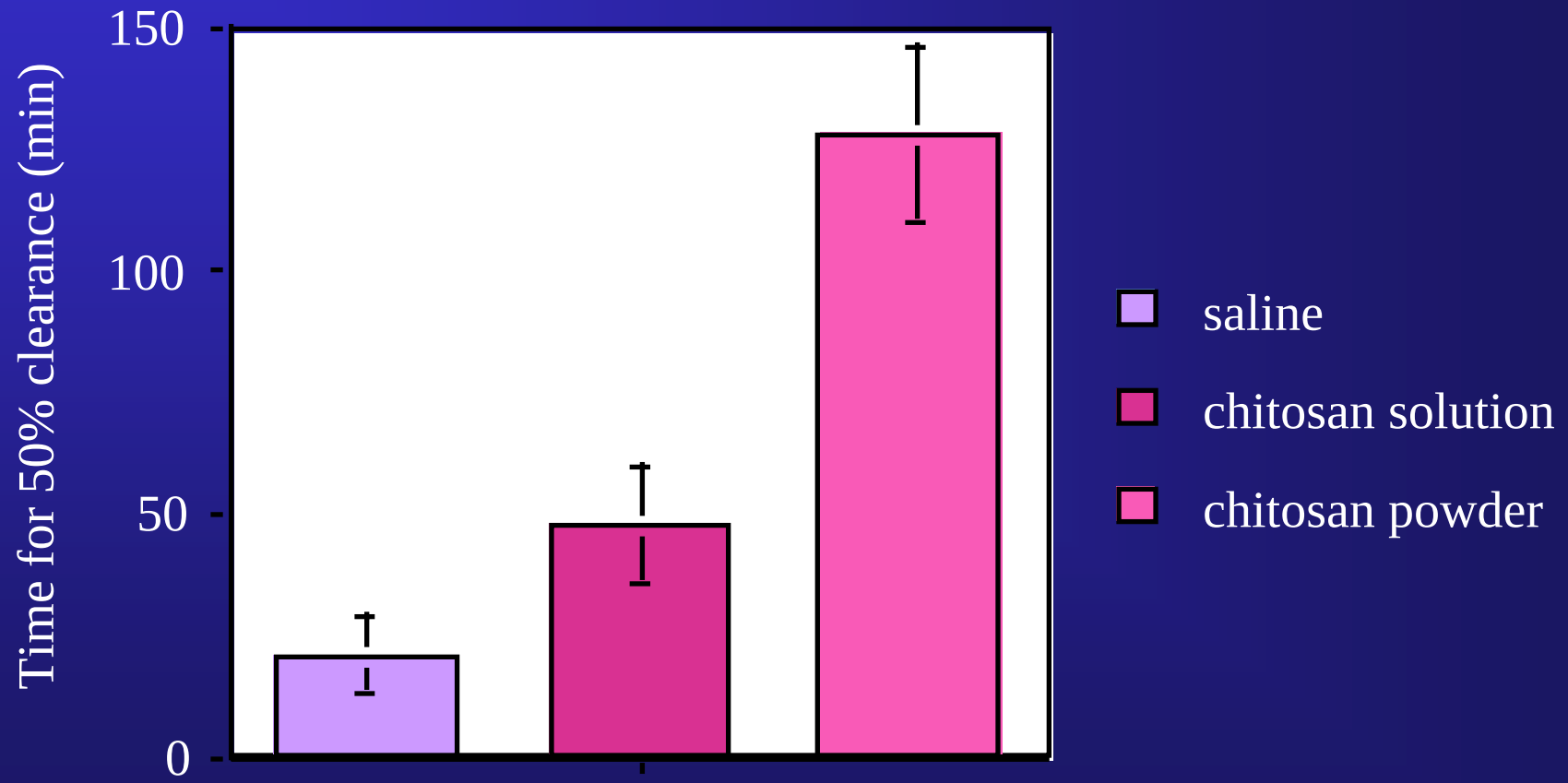


Mucoadhesion in the nose

Chitosan and nasal delivery

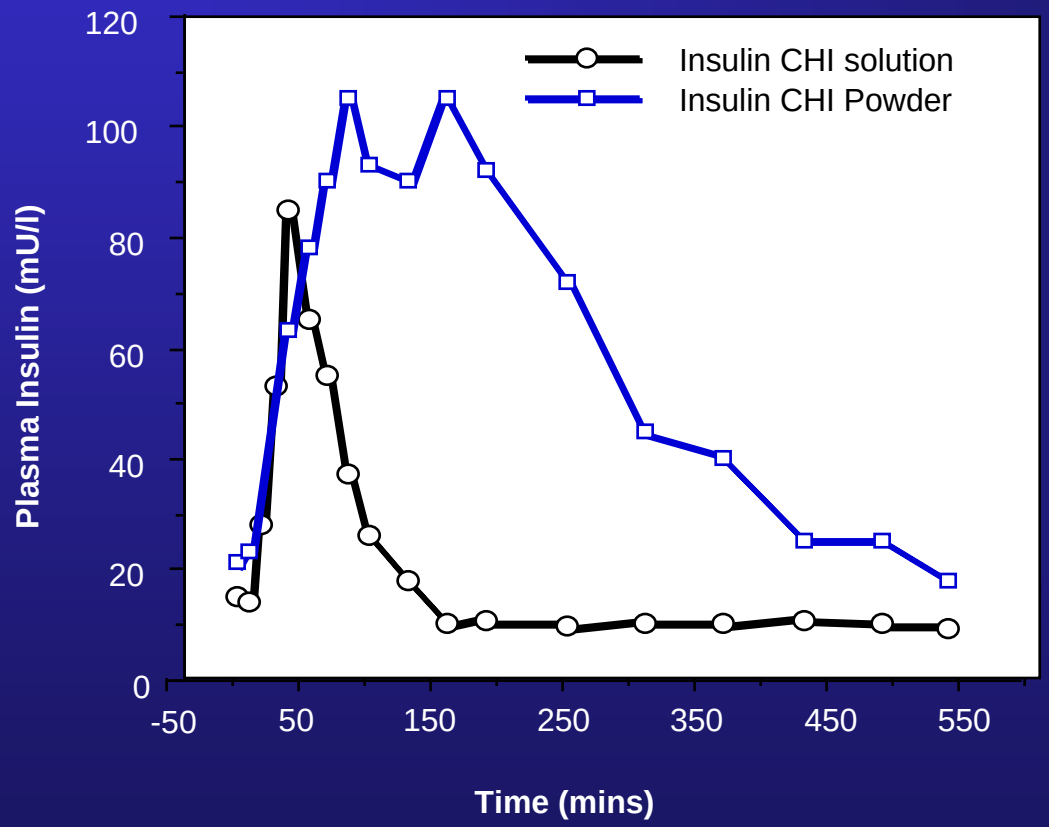
- Decreased Clearance Rate
  - chitosan is a mucoadhesive material
- Effect on inter-cellular transport
  - transient opening of “tight junctions” has been shown in cell cultures

Clearance of Chitosan Formulations from the Nasal Cavity of Man (n=4)



Illum , L. et al, 2002

Nasal administration of Insulin to Sheep with chitosan



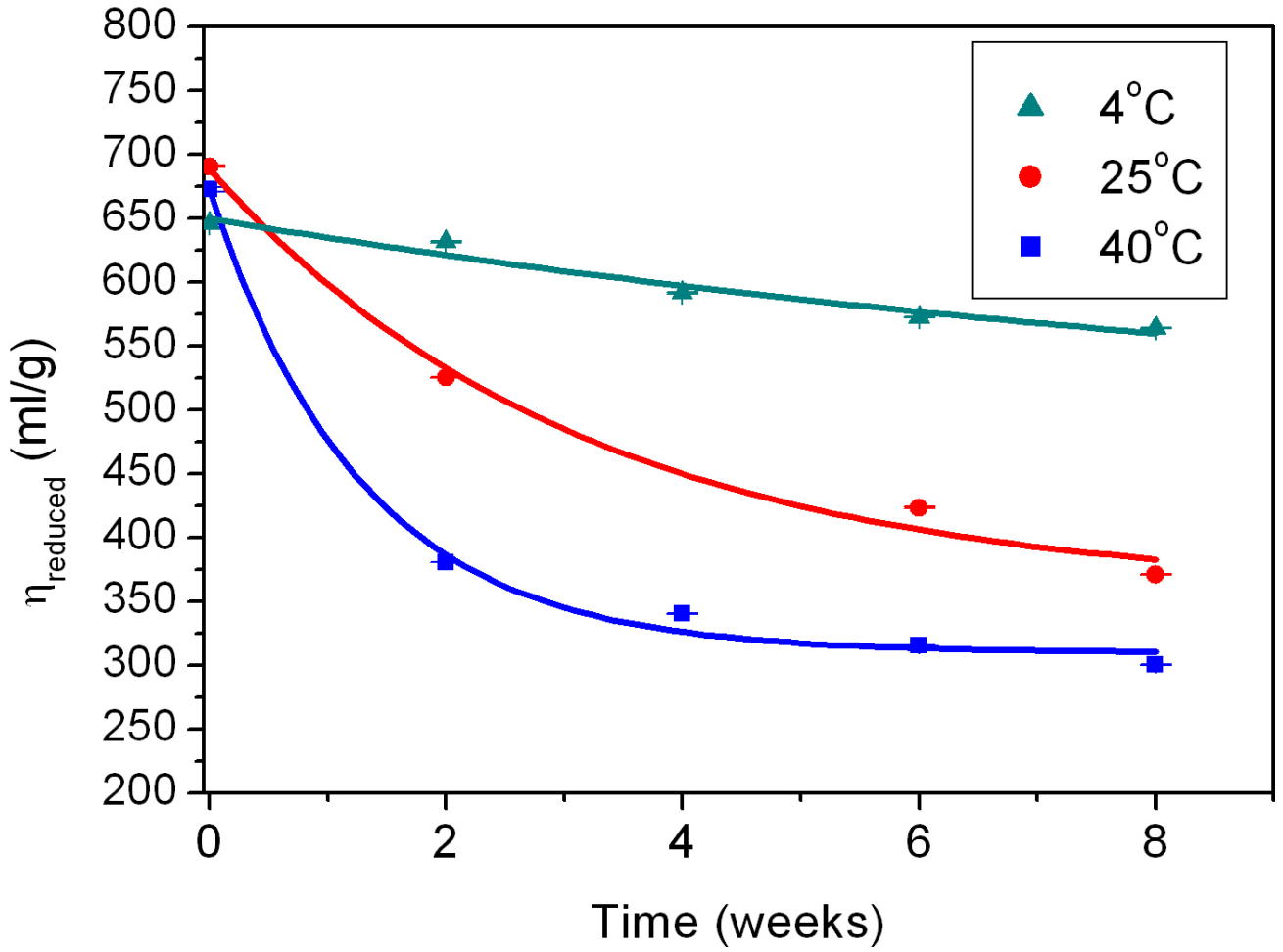
Illum, L. et al, 2002

Mucoadhesion: work in progress

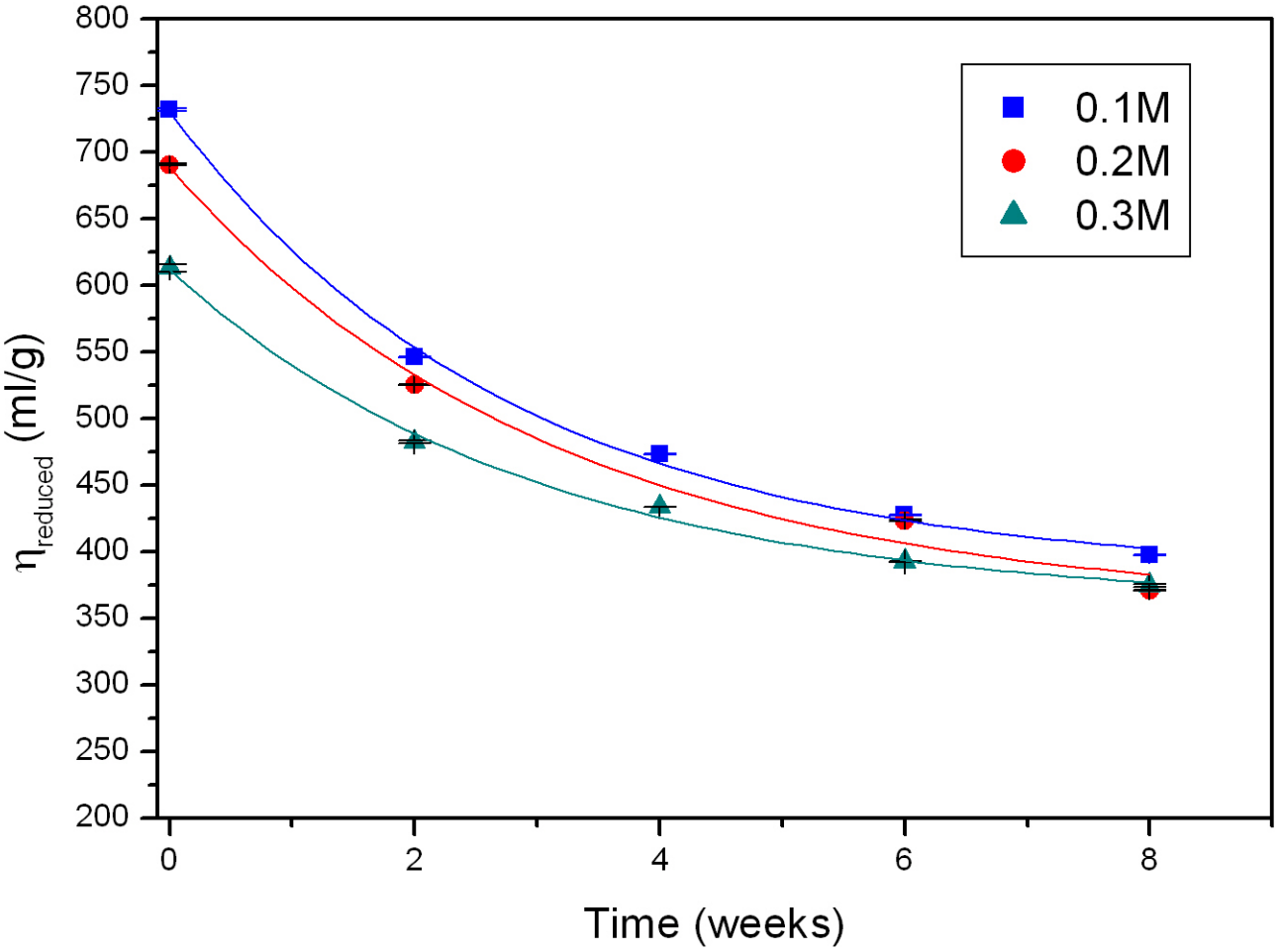
- **Efficiency of chitosan based encapsulation systems**
- **Stability of chitosan**

STABILITY OF CHITOSAN FORMULATIONS – viscosity is good here!

Chitosan: CL210 (F_A = 0.18), Effect of temperature on storage



Chitosan: CL210 (F_A = 0.18), Effect of ionic strength on storage



Fee, M. et al (2005)


Danke! - Thank you for your attention!

Some references

1. Harding SE (2006) Trends in Mucoadhesive Analysis. *Trends in Food Science and Technology*, 17, 255-262.
2. Harding SE (2003) Mucoadhesive Interactions, *Biochem. Soc. Trans*. 31, 1036-1041.
3. Deacon MP et al (2000) Atomic force microscopy of gastric mucin and chitosan mucoadhesive systems. *Biochemical Journal*, 348, 557-563.
4. Harding SE et al (1999) Biopolymer Mucoadhesives, *Biotech. Genetic Eng. Rev.*,16, 31-86.
5. He P, Davis SS, Illum L. (1998) *In vitro* evaluation of mucoadhesive properties of chitosan microspheres. *Int J Pharm.* 166, 75-88.
6. Deacon MP et al (1998) Structure and mucoadhesion of mussel glue protein in dilute solution, Biochemistry 37, 14108-14112.
7. Harding SE (1989) The macrostructure of mucins in solution. *Adv. Carb. Chem. Biochem*. 47, 345-381.

All refs (apart from #5) can be accessed from
http://www.nottingham.ac.uk/ncmh/harding_publish.html