Question One: What is a polymer?

Polymer: ‘A substance composed of macromolecules’ [IUPAC (International Union of Pure and Applied Chemistry)]

Macromolecule: ‘A molecule of high relative molecular mass, the structure of which essentially comprises the repetition of multiple units derived, either actually or conceptually, from molecules of low relative molecular mass.’

All polymers share a set of properties that make their study a valid thing in itself, whatever their chemical basis, because their physical properties are dominated by their size rather than their chemistry. While some macromolecules (e.g. DNA, proteins) have interesting chemical properties, most macromolecules even in vivo are used for their physical properties.

Natural Polymers
Synthesised in vivo

Nucleic Acids
Monomers = Nucleotide Phosphates (4) Polymers = DNA and RNA
Made by templating to existing molecules in a controlled environment.

Proteins
Monomers = Amino Acids (20) Polymers = Proteins and Peptides
Made by a complex process involving a nucleic acid template and a large number of functional proteins/nucleic acids.
Glycoprotein = protein with some saccharides attached (very common in eukaryotes)

Polysaccharides
Monomers = Monosaccharides (~24) Polymers = Starch, Cellulose, Hemicellulose, Hyaluronic Acid, etc.
Made by a series of functional proteins (enzymes)

Glutenin
Enormous proteins in wheat responsible for physical properties of wheat dough
Composed of LMW (285 amino acids) and HMW (581 amino acids) subunits joined together by disulfide bonds
**Poly(glucose)**

**Cellulose** made up of cellobiose – two glucose units joined by a $\beta$-1,4 link

Starch made up of maltose – two glucose units joined by an $\alpha$-1,4 link

![Chemical structures of $\beta$-1,4 and $\alpha$-1,4 linkages](image)

Geometry of these dimers determines conformation of polymer – ‘pleated sheet’ for cellulose vs. helix for starch – this in turn determines properties – elasticity, solubility, etc. Chemical modification can disrupt supramolecular structure – e.g., hydroxymethylcellulose is a water-soluble thickener used in diet foods.

**Starch** comes in two forms – straight chain amylose and hyperbranched amyllopectin. Amylopectin is synthesised with one reducing (opening) end and many non-reducing ends – straight chain segments called A, B, and C chains. *In vivo*, these molecules are packed together in a granule with a complex supramolecular structure. Ratio of amylose:amylopectin determines physical properties of starch.

When starch is cooked, the granules swell and soften and the amylose component is initially leached out. Eventually granules rupture, and viscosity of solution goes up since it is now a mass of entangled molecules. As solution is cooled, the free chains form intermolecular helices, giving a gel. This process of forming helices (‘retrogradation’) makes the product stiffer and continues over days: responsible for staling of bread.

**Enzymatic Modification of Starch**
- Chop up to make sweeteners, thickeners (amylases)
- Make more branched to make reversibly gelling starch (amylomaltases)
- Make less branched to make less digestible (debranching enzymes)
- Make into cyclodextrins for drug delivery, odour removal, etc. × $10^4$ $\$ value

**Lignin**
Polymer of phenols – three-dimensional cross-linked structure that makes hardwoods hard – degraded to form humic acid and fulvic acid, major soil components

![Chemical structures of lignin monomers](image)
**Poly(hydroxyalkanoates)**

Cellulose and lignin are **structural** polymers. Starch, by contrast, is an **energy-storage** material. Another example of a material for storing excess carbon are the polyesters known as poly(hydroxyalkanoates). These are possible replacements for poly(ethylene) and poly(propylene) and both bacteria and higher plants have been genetically engineered to produce them in quantity.

**Poly(isoprenoids)**

Only natural polymers without heteroatoms in the backbone. Examples are natural rubber (cis-poly(isoprene) from *Hevea brasiliensis*) and the related polymers gutta percha (trans-poly(isoprene) and chicle (random poly(isoprene)).

**Question Two: How are Polymers made?**

The two main mechanisms of polymer synthesis are **addition (chain) polymerisation** and **condensation (step) polymerisation**. There are problems with these as names, but as long as you know what they actually are you will be all right.

**Condensation (Step)**

So-called because most of these reactions produce water. e.g.,

\[
\text{Amine} + \text{Carboxylic Acid} \rightarrow \text{Polyamide (Nylon 6,6)} + H_2O
\]

Each of the COOH ends on the amide can react with NH₂ and each of the NH₂ with COOH, so the molecule will just get bigger and bigger. Each chain end is reactive, and chains start small and steadily grow. While most monomer is consumed very early, it is not until late in the reaction that very high molecular weights are achieved. Early in the reaction, only short polymer chains will be found, and late in the reaction only long polymer chains will be found. These reactions are relatively slow, generate relatively little heat, and are (in general) reversible under typical reaction conditions.

**Some more examples:**

- **Amide** (amine + carboxylic acid/acid chloride): \( \text{RNH}_2 + \text{R'}\text{COCl} \rightarrow \text{RNH(CO)R'} + \text{HCl} \)
- **Ester** (alcohol + carboxylic acid/acid chloride): \( \text{ROH} + \text{R'COOH} \rightarrow \text{RO(CO)R'} + \text{H}_2\text{O} \)
- **Urethane** (alcohol + isocyanate): \( \text{ROH} + \text{R'NCO} \rightarrow \text{R'NH(CO)OR} \)

Note that essentially the same polymer can be generated in a number of different ways - by reaction of a monomer with AA functionality with one with BB functionality, reaction of a monomer with AB functionality, or rearrangement of a monomer which already has the A+B functionality:
Addition (Chain)
So-called because it involves a rapid chain reaction of addition of monomers with a specific functionality (usually C=C) to an active centre (usually a radical, cation, or anion).

\[
\begin{align*}
\text{poly(styrene) radical} & \quad + \quad \text{styrene} \quad \rightarrow \quad \text{poly(styrene) radical}
\end{align*}
\]

Only the radical ends on the polymer can react with the vinyl groups, so once it is gone the chain will stay the same length forever. These reactions happen very fast, so some monomer will be incorporated into very long chains even while 99.9% of the monomer is still around. High molecular weights can be obtained in the very first moments of the reaction, and residual monomer may remain at the end. These reactions are relatively fast, can generate a lot of heat, and are (in general) irreversible under typical reaction conditions.

Mechanism of Addition Polymerisation
While condensation is the simple, step-wise repetition of essentially the same reaction over and over and over again, addition polymerisation can only be understood in terms of a number of different reactions.

First is \textit{initiation}, the generation of the active centre. For example, in free-radical polymerisation a free-radical may be generated by the thermal or photochemical decomposition of a peroxide or an azo-compound, then add to a monomer molecule:

\[
\begin{align*}
R-\text{N}=\text{N}-R & \quad \rightarrow \quad 2R• + N_2 \\
R • + M & \quad \rightarrow \quad RM • 
\end{align*}
\]

Next is \textit{propagation}, the rapid addition of monomer to active centre.

\[
\begin{align*}
\sim M_n• + M & \quad \rightarrow \quad \sim M_{n+1}•
\end{align*}
\]

A particular growing chain may lose its active centre in a number of ways. The loss of the active centre is \textit{termination} – for example, by combination of two radicals to form a bond.

\[
\begin{align*}
\sim M_n• + \sim M_m• & \quad \rightarrow \quad \sim M_{n+m}
\end{align*}
\]

The active centre may also \textit{transfer} from one molecule to another. In free-radical polymerisation, this occurs when the free-radical abstracts an atom from another molecule, such as a thiol chain-transfer agent.

\[
\begin{align*}
\sim M_n• + R'SH & \quad \rightarrow \quad \sim M_nH + RS•
\end{align*}
\]

The newly radicalised molecule may then start another chain...

Copolymers
If two or more monomers that can both polymerise by the same mechanism are brought together, they can both be incorporated into the same polymer, which is then called a copolymer. If we call the monomers A and B, the macromolecules of copolymer can be:

\textit{random} (\ldots ABAAAAABBBAAAAABAAABBAAB\ldots )

\textit{alternating} (\ldots ABABABABABABABABABABA\ldots )

\textit{block} (\ldots AAAAAAAAABBBBBBB\ldots )
Reacting monomers which have multiple reactive groups can lead to branched polymers, with the limit of a single gel molecule occupying the entire volume – e.g., the copolymerisation of acrylamide and methylene bisacrylamide:

The proportion of these two comonomers will determine the pore size and strength of the resulting gel.

**Question Three: What’s going on?**

**Polyisoprenoid Physical Properties**

(i) *Random polymer* (free-radical polymerisation)

Amorphous solid: No long range order. Cohesive Strength: 0.34 MPa, Tensile Strength: 10.35 MPa (100 °C)

(ii) *Natural Rubber* (trees)

Semi-crystalline solid: Helical secondary structure; “Strain-hardening”. Cohesive Strength: 1.37 MPa, Tensile Strength: 20.0 MPa (100 °C)

(iii) *Gutta Percha* (other trees)

Semi-crystalline solid: *trans* gives more extended chain than *cis* – secondary structure of pleated sheets. Brittle at room temperature. Cohesive Strength: High, Tensile Strength: Low

**Supramolecular order** also important - Fully crystalline poly(isoprene) would be far too brittle. Crystallites exist separated by amorphous regions – if the crystallites are too small, the polymer will be rubbery and weak; too large, and it will be strong but brittle. Pure synthetic poly(*cis*-isoprene) can be made using Ziegler-Natta catalysis, but is weaker than Natural Rubber; the supramolecular order in natural rubber latex particles provides appropriate crystallite size.

**Polymer Tacticity**

Whenever there are chiral centres in a polymer molecule, stereochemical isomerism can become important. Most monomers will give rise to chiral centres.

poly(hydroxyvalerate) poly(styrene)

In biological polymers, the stereochemistry of the monomer is important – only the correct stereochemistry will be biologically active. More generally, it is not the absolute stereochemistry that will affect physical properties, but the relationship between the stereocentres of adjoining centres. There are three limiting cases:

**Isotactic**

```
R R R R R R R R R R
```

**Syndiotactic**

```
R R R R R R R R R R
```

**Atactic**

```
R R R R R R R R R R
```
The line shown is the plane defined by the zig-zag backbone of the polymer,

**Molecular Weight Distributions**

Most structural polymers do not have a single molecular weight, but a distribution of molecular weights. This irritated organic chemists in the 19th century, since it seemed like a large step backwards. The molecular weights may be clustered around a single peak (unimodal) or several peaks (multimodal). The degree of variation in molecular weight can be expressed by a number called the polydispersity (\( = 1 \) for a monodisperse polymer).

Polydispersity = \( M_w/M_n \) where:

\[
M_w = \frac{\Sigma (M^2 \times n)}{\Sigma (M \times n)} \quad M_n = \frac{\Sigma (M \times n)}{\Sigma n}
\]

**Polymer Cohesion**

Polymers are solids even if they are made up on materials that have very weak intermolecular bonding – e.g., ethylene and butadiene are gases at room temperature. This is because there are a lot of weak intermolecular bonds in these materials. It is entropically unfavourable to break them all at once – so much so that many polymers cannot be melted at all, decomposing first. However, most show another phase transition at a much lower temperature- the transition from a glassy to a rubbery state.

**The Glass Transition Temperature (T_g)**

How rapidly a polymer can respond to stress depends on how easily chains can move past each other, and this is strongly related to the possibility of rotation about the backbone – which enables pendant groups to get out of each other’s way. Below the temperature where this becomes possible, a polymer will be brittle and glassy; above this temperature it will be rubbery.

This is clearly dependent on structure- anything that hinders rotation will increase the \( T_g \); anything that assists it will reduce the \( T_g \). Bulky side groups close to the backbone, stiffer backbone structure, and strong intermolecular bonding all increase \( T_g \), while long ‘floppy’ substituents that can act as reservoirs for thermal energy decrease \( T_g \). \( T_g \) can also be reduced by adding a low molecular weight ‘plasticiser’.

The dependence of glass transition temperature on Molecular Weight is given by the Flory-Fox equation:

\[
T_g = T_{g\infty} - \frac{K}{M}
\]

where \( K \) is a constant, approximately \( 10^5 \) for polystyrene
And \( T_{g\infty} \) is the \( T_g \) at very high molecular weight (M)

The dependence of glass transition temperature on copolymer composition is given (for a random copolymer) by the Fox equation:

\[
\frac{1}{T_g} = \frac{w_1}{T_{g1}} + \frac{w_2}{T_{g2}}
\]

where \( w_1 \) and \( w_2 \) are the weight fractions of polymers with \( T_g \)'s \( T_{g1} \) and \( T_{g2} \).
Because polymer molecules are so big, they are never fully crystalline and never fully unentangled (except in dilute solution!) – because of this, they will never form liquids that are as liquidy as small molecular weight species do, not solids that are as solidy.

**Elastomers**

The ‘springiness’ of rubber tyres, bands, erasers, etc., is typical of a class of polymers called elastomers. Characteristic of these materials is their ability to ‘bounce back’ after being stressed, to regain their original shape. Elastomers either contain **covalent bonds** between molecules, or **entanglements** where the chains are snarled together enough that they cannot become unsnarled during the time a stress is applied to the material.

**Rheology**

If you take a piece of polymer and apply a force (stress) to it, it is likely to deform in the direction the force is applied (strain). The slope of a plot of stress vs. strain is called the Young’s modulus and is characteristic of a particular material at a particular temperature. Below the Tg, you need to apply a lot of force to extend a polymer a little bit (large Young’s Modulus). Above the Tg, only a little force can extend a polymer a lot (small Young’s Modulus).

If the stress is not constant, but oscillating, the response of the material can be in phase with the stress (typical for a solid) or out of phase (typical for a liquid). Polymers usually show behaviour in between these two extremes, with a peak strain that is displaced somewhat from the peak stress. Under these conditions, the modulus is best expressed as a complex number, G, where:

\[
\text{Stress} = \text{Strain}[G'(\omega)\sin(\omega t) + G''(\omega)\cos(\omega t)]
\]

\( G' \) = Storage modulus : SOLID-like behaviour \( G'' \) = Loss modulus : LIQUID-like behaviour

If \( G'' > G' \), the polymer shows more liquid-like behaviour, and this is more likely at lower molecular weights. The high the molecular weight and the lower the density of the monomer unit, the higher the absolute value of G.

**Effect of Branching on Solid-State Properties**

Linear polymers are the simplest to represent mathematically and the easiest to understand – but in practice, branched polymers are very common and have a number of effects on physical properties.

1. Branches disrupt packing, leading to a more amorphous polymer and hence to a lower Tg and decreased storage modulus

*The science of how things flow*
(2) Branches can either increase or reduce the degree of entanglement; While long chain branches increase entanglements – making the polymer more elastomeric and increasing the storage modulus, short branches increase the amount of polymer between entanglements and the difficulty with which it can be stretched – which increases both loss and storage moduli.

Branching is extremely important to the drawing of fibres or films from molten polymers. At a particular molecular weight, the more long chain branches, the more stress is needed to break a drawn fibre. Conversely, lots of short chain branches will reduce the degree of entanglement and make a weaker fibre.

For poly(ethylene), the strength of the melt is inversely related to the strength of the solid polymer – Low Density PE > Linear Low Density PE > High Density PE (melt strength)

**Question 4: What do polymers look like in solution?**

We have already talked about entanglement, which suggests polymers are not rigid straight-line objects, but are more like pieces of string. Polymers are, indeed, more like pieces of string. In a solvent, polymers will be like pieces of string wiggling around in three dimensions. As a solvent becomes better for a polymer, it will become more expanded; as it becomes worse, it will shrink down towards a solid glob.

![Random Coil Diagram](https://example.com/random_coil_diagram.png)

Though the exact conformation of a particular molecule is unpredictable, always changing and very complicated, the general conformation of the ‘average molecule’ can be estimated – and used to explain a lot of things – using the **random coil** approximation.

**Random Coil** = The bond length between two monomer units is fixed, but its direction is completely random. No doubt you can see some errors in this approximation already. This gives you a random walk in three dimensions, leading to the following expression for the distance between the ends of the polymer chain:

\[
<r> = n^{1/2} \times l
\]

where \( <r> \) = root-mean-square distance of separation, and \( n \) is the number of bonds of length \( l \) in the chain.

In practice, this number must be multiplied by a constant \( C \) which takes into account the fact that bond angles are fixed, that two atoms can’t occupy the same space at the same time, and that the polymer may be more or less happy to be dissolved in a particular solvent.

**Coil-to-Globule Transition**

How happy a polymer is to stay dissolved in a solvent depends on the temperature. Being dissolved is usually entropically favoured, so will become more likely at higher temperature. Thus, most polymers have a **Lower Critical Solution Temperature** below which they adopt a phase-separated globular configuration.

* To get really anthropomorphic for a little while.
Some polymers also show an **Upper Critical Solution Temperature**, above which they become globular again. This is also driven by entropy and arises if the polymer induces a strong degree of order in the solvent. e.g., N-isopropyl acrylamide, which collapses at 32 °C to free water bound to the polymer...

The **Volume** occupied by a polymer chain (whether as a globule, or as a random coil including its entrained water) is related to the **Viscosity** (resistance to flow) of a solution of that polymer. There is a very solid theoretical relationship between them and any instrument that measures one of these quantities can give an idea of what the other will be.

**A few definitions:**

**Specific Viscosity** ($\eta_{sp}$) is the ratio between the viscosity of a solution and the viscosity of the solvent. It can be empirically determined by measuring the time it takes solutions to flow through a capillary.

$$\eta_{sp} = \frac{t - t_0}{t}$$

The limit of $\eta_{sp}$ divided by concentration as concentration goes to zero is the **Intrinsic Viscosity** ($[\eta]$). This can be related to the size and mass of a spherical particle by an expression derived by the little-known scientist Albert Einstein – it is directly proportional to volume, and inversely proportional to mass. $[\eta] \propto \frac{R^3}{M}$

A random-coil must be approximated as an equivalent sphere. The radius of this sphere is directly proportional to the distance between the ends of the polymer chain, and thus $[\eta] \propto \langle r \rangle^3 / M_v^*$. The volume of this sphere is called the **hydrodynamic volume** of the polymer molecule.

Of course, $\langle r \rangle$ should be dependent on $M^{1/2}$, so you might expect that in general $[\eta] \propto M^{1/2}$

This is true, but only for what is called a **theta solvent** – that is, when there are no attractive or repulsive interactions between parts of the polymer chain and other parts of the polymer chain. Empirically, the Mark-Houwink(-Kuhn-Sakurada) relation is very important:

$$[\eta] = K \times M^a$$

Here $K$ and $a$ are the ‘Mark-Houwink Parameters’. If $a < 0.5$, interactions between units in the polymer are attractive; if $a > 0.5$ they are repulsive. $K$ and $a$ are sensitive to solvent and temperature, as you might expect, and the relation is valid only above ~ 100 monomer units where the random coil approximation is valid.

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*M_v is the viscosity-average molecular weight, for a typical polymer with more than one molecular weight present. Its relationship to the other two averages will vary, but it is usually near $M_w$.***
Question 5: Can you tell us a lot more things about poly(acrylamide) gel that might well be on the exam?
- Used to separate proteins and DNA in PAGE (PolyAcrylamide Gel Electrophoresis)
- PAG used in PAG usually Copolymer of acrylamide and methylene bisacrylamide (typically 80:1 to 20:1)
- ..and typically 8 - 20 % polymer
- Lots of crosslinker, lots of polymer = small pores
- made by Free-radical polymerisation with initiator typically ammonium persulfate, with tetramethyl ethylene diamine to accelerate decomposition.

Question 6: How does Size-Exclusion Chromatography work?
While PAGE gels are usually prepared in situ, size-exclusion chromatography (SEC) uses columns which are prepacked with similar gels. Numbers that come out of these machines are very frequently reported as molecular weights, but in fact SEC does not measure molecular weight. Neither does Ultracentrifugation, Dynamic Light Scattering, or Viscometry. All of these separate molecules by hydrodynamic volume, or by some combination of hydrodynamic volume and mass.

In Size-Exclusion Chromatography, the size of the chains determines their fate as they pass through the column filling, a crosslinked porous material with a distribution of pore sizes. Smallest chains can go in all the pores, have a large volume to explore, and thus elute last. Largest chains never go in any pores, have the smallest volume to explore and thus elute first.

Three things are self-evident:
- All the very small molecules will come out at the same time.
- All the very large molecules will come out at the same time
- There is no reason for there to be a nice simple relationship between time and size for the molecules in between.

The (hopefully) linear region in between must be calibrated using polymers of known volume – calibration will be universal for all objects of that volume (assuming various conditions are met).

Getting from hydrodynamic volume to molecular weight is straightforward for a linear polymer with known Mark-Houwink parameters – but for branched polymers, it is a teensy bit more complicated. A branched polymer will always have a lower hydrodynamic volume than a linear one of the same molecular weight.

Question 7: How is all this information about properties of polymers in the solid state and in solution relevant to biopolymers?

Relation between biopolymer structure and function
Firstly, it is worthwhile to consider the different structures which a polymer can adopt, and how changes in monomeric units can effect the structure. We have already done this for synthetic polymers in a limited fashion, and now we will look at some typical structural biopolymers.

In protein modelling, all bond angles are considered to be fixed, and the conformation of a monomer unit can be specified by considering only
the angles of rotation about two bonds (\(\phi\), about the C\(_{\alpha}\)-N bond, and \(\psi\) about the C\(_{\alpha}\)-C(O) bond.

Actual conformations of protein residues cluster around a few areas of a plot of \(\phi\) vs. \(\psi\), and where a number of successive residues have the same conformation an ordered **Secondary Structure** will be seen. The most important of these are the **Alpha Helix** and **Beta Pleated Sheet**.

**Alpha Helix**
A single-chain helix held together by hydrogen bonding, 0.54 nm per turn, 0.15 nm per amino acid residue. The ends are prone to unravel, so need to be composed of strongly hydrogen-bonding ‘capping groups’ if the structure is to be preserved (S, D, E).

Amino acid composition has further effects on stability of an alpha-helix:
- Big substituents destabilise (Y, W, F, M, I, V, T, C)
- P (proline) causes kink in chain
- E (aspartate) and N (asparagine) disrupt main-chain hydrogen bonding

**Beta Pleated Sheet**
A multi-chain secondary structure where chains are held together by hydrogen-bonding in a sheet.

These structures tend to be less sensitive to composition than the alpha-helix, as bulky substituents point away above and below the plane.

**Tertiary Structure** = Entire Three-dimensional configuration of a protein chain
**Quaternary Structure** = Complete protein structure, including multiple chains, metal ions, etc.

These are determined by the monomers and in turn determine the properties. Just as in synthetic polymers, it is the structure rather than the particular monomers that build it that is most important.
Example Structural Protein #1: Collagen

This constitutes about 25% protein in most vertebrates, and is found in bones, skin, blood vessels, tendons, cornea. It is composed of rigid, rod-like protein aggregates about 300 nm × 1.5 nm (Tertiary Structure) which are cross-linked in a variety of different ways to give different fibres (Quaternary Structures) suitable for these various in vivo applications.

Collagen is rich in glycine, which occurs at every third residue throughout most of the chain, and also in proline (about 25%) and the modified amino acids hydroxyproline and hydroxylysine (about 25% together). Each of these amino acids has a particular function in generating the Secondary, Tertiary, and Quaternary structures of collagen.

The Secondary structure of collagen is a left-handed helix with three residues per turn – this is not an alpha-helix, as it contains far too much proline. This same high amount of proline makes the chain unusually stiff for a protein (remember what we said about Tg in relation to synthetic polymers – rings in backbone make strands more rigid!).

Three of these chains then associate together to form a tropocollagen fibre. The most common form has two chains of one sequence (α1) and one of another (α2). Only glycine is small enough to allow the chains to approach close enough to form this triple helix. In order to maintain the association, however, the contribution of another amino acid is required – hydroxyproline, which greatly strengthens intermolecular hydrogen bonding. Without hydroxyproline, chains do not associate. Parenthetically, ascorbic acid (vitamin C) is required for proline hydroxylase to function, hence the symptoms of scurvy such as bleeding gums and joint pain caused by the inability of the body to generate new collagen where it usually has a high turnover rate.

Finally, aggregating these tropocollagen fibres into resilient higher-order structures requires strong hydrogen bonding between fibres. In addition, a degree of cross-linking occurs which greatly increases the strength and elasticity of the fibre. The amino acids essential for this are lysine and hydroxylysine, which can be oxidised to aldehydes in vivo by lysine oxidase...

...which then react with un-oxidised lysine to give a covalent bond between chains.
This cross-linking occurs both between strands in the triple helix of tropocollagen, and between adjacent triple helices in the supermolecular microfibril structure. Corneal collagen is more crystalline and more highly cross-linked collagen found elsewhere in the body.

**Example Structural Protein #2: Elastin**

Elastin is the polymer which – surprisingly, given its name – is responsible for most of the elastic properties of bits and pieces of vertebrates. In humans, it comprises 2-3% of the skin, 30% of the aorta, and about 50% of elastic tendons. While collagen tends to form ordered fibrils, elastin is much more likely to form an extended elastomeric ‘net’.

It is rich in glycine (30%) and in hydrophobic amino acids, which form random hydrophobic coil regions, and in lysine, which forms lysine-rich hydrophilic regions. So it can be considered a ‘block copolymer’ of hydrophobic and hydrophilic monomers.

When numbers of tropoelastin molecules are expressed, the hydrophobic regions associate together, and the hydrophilic regions associate together. Mere stress is unable to pull the hydrophobic domains apart, so they act as ‘virtual cross-links’ in an elastomeric network. This is exactly the same motif (though with a different molecular basis) that is seen in the poly(styrene-butadiene) rubbers used in shoe soles.

Covalent cross-linking between lysine and allysine adds additional strength and controls the degree of elasticity. Note that lysine oxidase is this essential for the proper production of elastin as well – so wounds will not heal effectively without ascorbic acid. Elastin is astonishingly tough stuff and we keep most of it for our entire lives.

**Example Structural Protein #3: Keratin**

Unlike the last two examples, α-keratin (the main polymer in hair and horn) is almost entirely alpha-helical in secondary structure. In addition, it is very rich in cysteine (up to 15%). It is cross-linking between cysteine groups to form the cystine species with its –S–S– that is responsible for the hardness of keratin.

Wool, for example, has relatively light cross-linking, while horn is heavily cross-linked.

Mammals have only α-keratin; birds and reptiles have β-keratin as well; this protein (found in scales and feathers) tends to have smaller side chains and mostly adopts an antiparallel beta sheet configuration.

**Fibroin**, the polymer found in silk, consists of a very regular beta-pleated sheet sequence with only methyl groups (from A) sticking out of one face and only hydroxy groups (from S) sticking out the other. These sheets are strong and resilient, but can slide past one another to give the ‘silkiness’ of silk...

**Glutenin**, the polymer that allows to make light, fluffy bread, is cross-linked by the same chemical links that cross-link hair. Instead of short alpha-helical sections very rich in cysteine, however, it consists of high molecular weight subunits bearing only a few cross-linking sites.

Researchers working with glutenin, keratin, and amylpectin all claim that they are working with ‘nature’s largest molecule’. But once you start cross-linking reactions, there is no theoretical limit.
Question Eight: Did we learn all there is to know about colloids last year?

You may recall that a colloid is a mixture of two phases, one of which is a **dispersed phase** consisting of pieces of approximate dimensions (5 nm – 10 microns diameter), and the other a **continuous phase** in which these particles sit. Different names are given to different colloids depending on the nature of the two phases – if both are liquid, it is called an emulsion; if the disperse phase is solid or liquid and the continuous phase gas, an aerosol; disperse solid and continuous liquid, a sol, etc.

Colloids show interesting properties typically because the disperse particles are about the size of a wavelength of light, and they have a much greater surface area:volume ratio than macroscopic substances.

Biologically, many materials are best understood as colloids.
- Blood = dispersion of cells
- Milk = dispersion of fat globules and casein micelles
- Cytoplasm = dispersion of water in protein matrix

**Milk**

This interesting colloid contains two different kinds of dispersed particle:

It is approximately 4% by weight fat, which exists in globules larger than 500 nm and reaching beyond the colloidal scale in unhomogenised milk; it is stabilised by lipids and proteins on the surface. Another ~4% of milk by weight is composed of casein micelles (80 – 300 nm diameter). One micelle is a mass of proteins (α,β,κ casein) held together by binding to calcium phosphate crystallites (9-20 nm diameter).

If you ultracentrifuge milk, the fats will go to the top, the casein micelles will settle to the bottom, and a greenish liquid containing lactose, salts, and proteins will remain in the middle.

**What keeps the suspended particles in suspension?**

Essentially, the force of gravity acting on the suspended particles is outweighed by:
- Weak thermal convection currents (bulk flow due to difference in T of top and bottom of milk)
- Brownian Motion (the motion given to particles by molecules running into them)

**Brownian Motion**

As molecules constantly hitting particles, they transfer kinetic energy. The molecule will traverse a random walk, where the mean end-to-end distance travelled \(d(t)\) will be given by

\[d(t) = n^{1/2} l\]

where \(n\) is the number of collisions in that time and \(l\) is the average distance the particle travels between collisions.

The larger a particle is, the more often it will be hit, and the shorter the distance it will travel between times it is hit – both because it is hit more times, and because the same amount of kinetic energy will not

The length of step in random walk, \(l \propto 1/\text{size}\)

The number of steps, \(n \propto \text{size}\)

\[\therefore \text{ Overall distance travelled is smaller the larger the particle}\]

Quantitatively, the following expression has been determined to hold - \(d^2(t) = 6Dt\)
Where $D$ is the diffusion coefficient, which for spheres is given by:

$$D = \frac{kT}{d^3 \eta}$$

Here $\eta$ is the viscosity of the continuous phase and $k$ is Boltzmann’s constant ($8.314/6.022 \times 10^{23}$, JK$^{-1}$)

There are a number of analytical methods for particle sizing which rely on determining this diffusion coefficient and then extrapolating a particle size- these include Ultracentrifugation, Dynamic Light Scattering, and Field Flow Fractionation.

**What keeps the suspended particles from clumping together until they are large enough that these convection currents and Brownian motion can no longer hold them up?**

It has been shown that uncharged particles subject to Brownian Motion will rapidly coagulate to remove surface free energy: $t_{1/2} \sim 1$ ms – 1 s for typical colloid particle number concentrations.

Particles can be stabilised by charging their surfaces, or by using a steric stabiliser.

**Ionic Stabilisation**

If you just put charge on the outside of the dispersed particles, electrostatic repulsion between particles will keep them apart. One way to do this in a sol or emulsion is to use a surfactant (e.g., sodium dodecyl sulfate, $\text{C}_{12}\text{H}_{25}\text{OSO}_3^{-} \text{Na}^{+}$ “SDS”).

Another example are the charged groups on proteins –proteins tend to fold so that their hydrophilic charged groups are on the outside, hydrophobic groups on inside.

In an aerosol, the interactions between the charged particles will be described completely by repulsion of the charged particles themselves- but in a sol, the participation of counter-ions is also important.

In SDS, note that there are enough Na$^+$ ions to balance completely the charge on the sulfoxylate anions. Some of these will stick close to the surface of the colloid (Stern Layer), while others will be distributed throughout the aqueous phase. But as you would expect, they are more likely to be found near the negatively charged surfaces.

Interaction between these clouds (of the opposite charge to the particle surfaces) is what really repels particles in most ionically-stabilised sols and emulsions.
This hand-wavy ‘electrical double layer effect’ has been quantified in what is known as DLVO\textsuperscript{*} theory, which accurately describes the behaviour of charged particles subject to Brownian Motion.

Adding more counter-ions can destabilise a colloid. Experimentally, the rate of coagulation is proportional to $z^3$, where $z$ is the charge on counter-ion…

Fewer $3^+$ than $2^+$ than $1^+$ ions are needed to cancel out colloid charge on negatively charged colloid, leading to a more compact counter-ion cloud.

Steric Stabilisation
Colloids can also be stabilised by absorption of a polymer with lyophobic/lyophilic groups on the particle surface. When two sterically-stabilized colloids are pushed together, chains are compressed and their local concentration is increased, both of which are entropically unfavourable. This is the role of some polysaccharide substituents on proteins.

Proteins and other electrolytes can also act as electrosteric stabilisers, where an adsorbed charged polymer acts by both mechanisms. These are resistant to traumatic events like freezing and thawing that can irreversibly destabilise colloids stabilised by other mechanisms.

Aggregation
The various means of stabilisation all generate an energy barrier to aggregation of two particles. Brownian motion is one way of getting over this barrier; but it is not the only one; it will be important only for the smaller size range of colloidal particles. 

Differential sedimentation = particles falling at different rate. It is important for large, high density particles.

Differential Shear = convection currents, any stirring that will move some particles faster than others. It is important for large, low density particles

Heating (which accelerates Brownian motion and convection currents), Agitation (increasing shear) and centrifugation (increasing sedimentation) can all therefore be used to speed up aggregation of a colloid.

The action of a counter-ion in destabilising colloids has already been mentioned. Both simple ions and polymeric ions (e.g., poly(quaternary ammonium) species) can be used. The other chemical means of destabilising colloids is to add a polymeric flocculant.

Flocculation is theoretically if not always practically reversible and uses high molecular weight (~$10^6$) water-soluble polymers. These bridging flocculants increase speed of settling and are very widely used in municipal and industrial water treatment, cheesemaking, sugar

\footnote{Named for its discoverers, Derjaguin and Landau in Moscow and Verwey and Overbeek in the Netherlands}
milling, etc. The process is dynamic, usually complete in seconds or minutes, and requires as little as a few ppm of flocculant.

![Diagram of milk emulsion](image)

1 μm

**More About Milk**

Milk has two different components, which are stabilised in different ways. It is possible, using principles of colloid science, to destabilise one component or another selectively, or all at once.

**Adding acid...**

protonates the lipids which stabilise the fat globules, leading to aggregation

decreases the surface charge on casein micelles (aggregation)

solubilises calcium phosphate in micelle structure so casein chains leave structure; as they do, they expand, increasing the viscosity of the continuous phase and possibly contributing to bridging flocculation.

**Renneting enzyme (chymosin)...**

cleaves κ-casein chains from surface of casein micelles, reducing their stability and leading to aggregation (cheese). Fat globules may be entrained, but are not destabilised.

**Shearing...**

brings about the coagulation of the large, non-dense fat globules (butter); has much less effect on smaller casein micelles.

**Adding salt** causes the aggregation of casein micelles, fat globules

**Heating** speeds the rate of aggregation, and also denatures whey proteins first, causing bridging flocculation. Eventually it will denature casein as well, leading to the fragmentation of the casein micelles.

Electrophoresis is the separation of particles by how they flow through a solution when placed in an electric field. The **electrophoretic mobility** is a function of mass ($\propto m^{-1}$) and charge at hydrodynamic shear plane ($\propto q$), which together will determine how fast the particles will move.

In **SDS-PAGE** (sodium dodecyl sulfate polyacrylamide gel electrophoresis) particles are all assumed to be covered with a uniform layer of surfactant, so mobility dependent on mass only...
These are important things to know:

- Polymers are made up of monomers
- They are less chemically reactive than monomers
- They share ‘polymer’ physical properties, whatever they are made of
- These properties are sensitively dependent on the way the monomers are put together (e.g., starch, cellulose)
- Sequence determines Structure determines Properties
- What ‘Chain Growth’ and ‘Step Growth’ mean, what their distinctive features are, and what monomers you might use for each of them.
- What Copolymers are and how to make them.
- Why natural rubber and gutta-percha have different properties, and what those properties are.
- What $M_w, M_n$, polydispersity, unimodal and multimodal mean
- What $T_g$ is, and how it is determined by structure
- What is an elastomer, and why
- The ingredients of a PAG and what role each one plays
- What instruments that measure ‘molecular weight’ really measure
- What Mark-Houwink-(Sakurada-Kuhn) parameters and a theta solvent mean
- What branching does to hydrodynamic volume
- How incorporation of different amino acid might affect the secondary structure and macroscopic properties of a protein
- The chemical basis for cross-linking in collagen, keratin, and elastin
- That the same macroscopic behaviour arises from the same kinds of organisation on the molecular level in both synthetic and natural polymers
- Why colloids are special
- How they are stabilised
- How they are destabilised
- The colloidal composition of milk and the different ways it can be destabilised
- How coagulation and flocculation work
- What SDS is for in SDS-PAGE
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