Drug Discovery (W1)

Drug Discovery and Prodrugs (L1)

Source of Drugs

- Drugs were originally derived from natural sources
- These natural resources still serve as important lead compounds for the development of new drugs
- Most new leads however are now synthesised in chemical laboratories
- **Four main** sources for new drug leads:

1. Natural products
2. Existing drugs - “Me too” drugs
3. Biological screens (Phenotype screening)
4. Physiological mechanisms (Target based approach)

Natural Products

- Provide the oldest source for new medicines
- Novel compounds have been created through the process of evolution and many are used in defence mechanisms
- Biologically active molecules are isolated from a huge variety of (not limited to);
  a. Plant sources
  b. Animal sources
  c. Marine sources
  d. Microorganism
- Plant based medicines traced back to Neanderthals
  - Analysis of ancient DNA from dental calculus shows that a Neanderthal with dental abscess self administered salicylic acid (birch tree bark) and penicillium Bacteria from moulded grasses

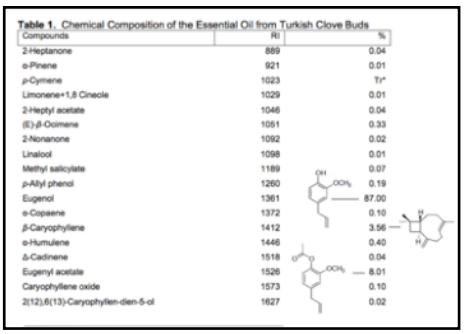


Table 1. Chemical Composition of the Essential Oil from Turkish Clove Buds

Compounds	RI	%
2-Heptanone	889	0.04
α-Pinene	921	0.01
p-Cymene	1023	Tr*
Limonene+1,8 Cineole	1029	0.01
2-Heptyl acetate	1046	0.04
(E)-β-Ocimene	1051	0.33
2-Nonanone	1092	0.02
Linalool	1098	0.01
Methyl salicylate	1189	0.07
p-Allyl phenol	1260	0.19
Eugenol	1361	87.00
α-Copaene	1372	0.10
β-Caryophyllene	1412	3.56
α-Humulene	1446	0.40
δ-Cadinene	1518	0.04
Eugenyl acetate	1526	8.01
Caryophyllene oxide	1573	0.10
2(12),6(13)-Caryophyllen-dien-5-ol	1627	0.02

Isolation of volatile oils from natural products and biological assay

- Syzgium aromaticus
- Native to Indonesia
- Folk medicine, food, flavourings, perfume
- Antimicrobial, anaesthetic, antioxidant

Natural Products

Plant sources

- Plant source is the oldest source of drugs
- Almost all parts of the plants are used i.e. leaves, bark, fruits, roots, etc
  - **Cinchona tree** (bark) - **Quinine**, Antimalaria
  - **Poppy plant** (flower) - **Morphine**, analgesic (pain killer)
  - **Madagascan rose periwinkle** - **vincristine**, anti-cancer

Animal sources

- Many of these natural products are extremely **toxic** (used as chemical defence) — lead drug
  - **Phantasmal poison frog** (Epipedobates tricolor) - **epibatidine**, analgesic
  - **Brazilian pit viper** (bothrops jararaca) - **teprotide** -> **captopril**, antihypertension agent
  - **Porcine pancreas** - **insulin**, diabetes
- Less richness of bioactive sources and animals can run away, don’t need to rely on chemical warfare. Greater source in plants as they can’t run away.

Marine sources

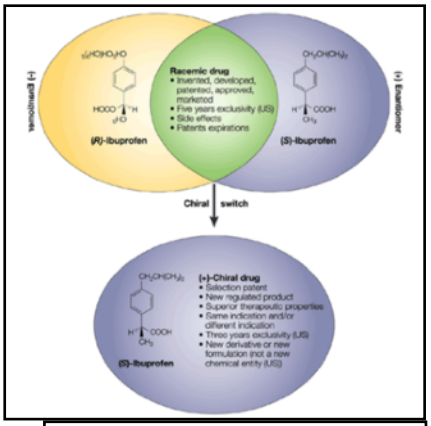
- **Cone snail** (Conus magus) - **omega-conotoxin MVIIA.** Synthetic - **Ziconotide**, Analgesic
  - This is a peptide, more quickly digested before entering the blood stream. Must be injected into spinal fluid to have effect
- **Caribbean tunicate** (Ecteinascidia turbinate) - **Ecteinascidin-743**, Anti-cancer
- **Ascidian** (Aplidium albicans) - **Aplidine**, Anti-cancer
- **Algae** (gambierdiscus toxins) - Ciguatoxin

Microorganisms sources
- **Penicillium fungi** - **penicillin**, antibiotic
- **Actinobacteria** - **streptomycin**, antibiotic
- **Oyster mushroom** - **lovastatin**, lowering cholesterol

Natural Products
- Many of these natural products are **extremely toxic** and are unsuitable for use as drugs
- **Synthetic chemistry** is used to **modify the activity** of these molecules
- Through **chemical modification**, Nature's original design can be altered to provide analogues with improved bioavailability, safety, potency and selectivity
- Example:
  - Conotoxins are extremely toxic
  - Omega-conotoxin MVIIA, Synthetic - Ziconotide
  - Analgesic - severe pain management
  - Must be administered intrathecally (i.e. directly into the spinal fluid)

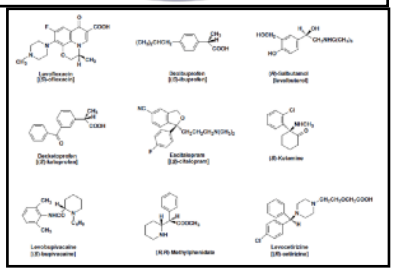
Existing Drugs - 'Me too' drugs
- Also provide a useful place to start the discovery of new drugs
- The cost of introducing a new drug to the market is extremely high and a very risky process
- To improve the chances of success and reduce costs, companies produce drugs with **similar structures and activities to their competitors**
- Modify the structure sufficiently such that to **avoids patent restrictions**, retain activity and ideally **improve therapeutic properties**
- This has been the most common and reliable route to NCEs (New Chemical Entities)
- Example:
  - Proton pump inhibitors - group of drugs - long-lasting reduction of gastric acid production
  - pH in the human stomach is ~1.4, problems with acid secretion - ulceration

'Me too' Drugs
- Most of the new drugs reaching the market today are **single enantiomers**, rather than the racemic mixtures
- Many of the new single-enantiomer drugs were developed as such, but there are also important examples of new single-enantiomer drugs derived from '**chiral switches**' of established **racemates**



Concept of Chiral Switches:
- Racemate undergoes chiral switch to produce a single-enantiomer that is more biologically active

Marketed single enantiomers of agents which have undergone the chiral switch:



What are the four main sources of drug leads?
- natural products, existing drugs - "me too" drugs, biological screens, physiological mechanisms

List the benefits of 'Me too' drugs
- Improve the chances of success, reduce costs

Define the terms below
- 'Me too' drugs: Drugs with similar or superior activities and molecular structure to those of the competitor

- Chiral switch: Chiral drugs that have already been claimed, approved and marketed as racemates or as mixtures of diastereoisomers, but have since been redeveloped as single enantiomers

Drug Discovery II (L2)

Drug discovery approaches

- Four main sources of new drug leads

1. Natural products
2. Existing drugs - 'Me too' drugs
3. Phenotype screening
4. Target based screening

Phenotype Screens

- Screening drugs against a biological target, looking for changes in its form or behaviour (phenotype)
- Phenotype screening can be
  - In vitro - e.g. Using cultured cell lines or tissue; quicker and less expensive
  - In vivo - e.g. whole animal based approach. Expensive, slow and ethically challenging. Often uses fruit flies, zebrafish or mice. Results are more relevant to disease

Phenotype Screens

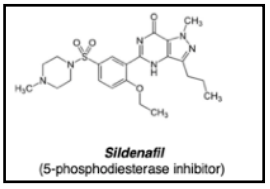
- A (bad) example of phenotype screen:
  - Dr Simpson and two of his assistants, used to sit every evening in Dr Simpson's dining room to try new chemicals to see if they had any anaesthetic effect. On 4 November 1847, they decided to try a ponderous material named **Chloroform**. On inhaling the chemical they found that a general mood of cheer and humour had set in. But suddenly all of them collapsed only to regain consciousness the next morning.
- A (better) example of a phenotype screen:
  - Phenotypic High-Throughput Screening elucidates target pathway in breast cancer stem cell-like cells

A (better) example of a phenotype screen:

1. Engineer cell line with required properties (cancer stem cells that could be easily cultured)
2. Screen cells against library of 300,000 compounds (qualitative screen - toxic or non-toxic). Identified 3200 'hits'
3. Removing hits with known toxicity left 2200 compounds
4. Compounds retested for potency (quantitative screen) and tested for toxicity against control cells
5. 26 compounds identified as >25 times more toxic to target cells than control
6. 2 compounds selected as suitable candidates
7. 53 analogues synthesised and tested

Phenotype Screens

- Sometimes it is possible to exploit a drug's side effects, e.g. Viagra
- This drug was originally being investigated for the treatment of angina (heart/ chest pain)
- It is now prescribed for the treatment of erectile dysfunction



Sildenafil
(5-phosphodiesterase inhibitor)

Sildenafil - Discovered by mistake

- Sildenafil as a treatment for angina
- The trials were unsuccessful and did little to dilate coronary arteries
- Common side-effect - improved erections while taking the pills
- This led to a dramatic shift in the direction of the research to the treatment of impotence
- Phenotype screening is making a comeback
  - Largely due to advances in in vitro cell culture technology and genetic engineering

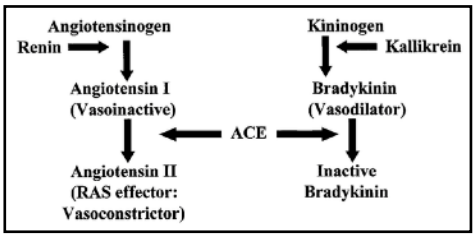
Target-based screening

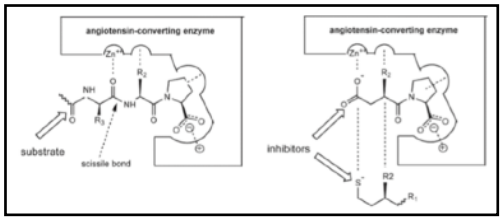
- A modern approach to drug design - modern technological developments and understanding of disease mechanism at a molecular level

- Has become the major route to lead discovery
- Firstly, the cause of the diseased state is identified, and a target is identified (critical enzyme, signalling pathway ect)
- An in vitro screen must be developed to test drug candidates against the target
- Requires close collaboration between chemists and biologists

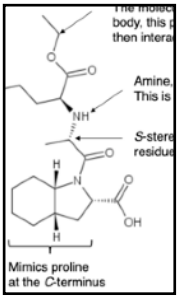
Target-based screening
- Five main sites for drug action
1. Enzymes
2. Cell replication and protein synthesis
3. Receptors
4. Transporter systems
5. Storage sites

Captopril
- In 1939, a brazilian pharmacologist named Mauricio Rocha e Silva began to study the effects of snake venom on animals
- In 1948, they identified **bradykinin**, a 9 amino-acid peptide that is released after a bite, and causes dilation of blood vessels and reduction of blood pressure
- **Angiotensin I**, an inactive decapeptide, is converted into A**ngiotensin II**, an octa-peptide with potent vasoconstrictor activity, and…
- **Bradykinin**, a nonapeptide with potent vasodilatory activity, is converted into an inactive heptapeptide
- An inhibitor of the converting enzyme would therefore result in lowering of blood pressure and be useful for the treatment of hypertension
- **BPP 5a** - greatest **in vitro** activity, pentapeptide
- **Teprotide** (Squibb and Merck) - greatest **in vivo** potency
  - Pro-Glu-Trp-Pro-Arg-Pro-Glu-Ile-Pro-Pro-OH
  - Resistant to hydrolysis
  - Poor oral availability





Perindopril (Solvay Pharmaceuticals-Abbott)
- The molecule is delivered as its ethyl ester to aid absorption
- Once in the body, this prodrug hydrolyses to the carboxylic acid. This functional group then interacts with the Zn2+ in the active site of the converting enzyme
  - Contains amine, not an amide. This is stable to hydrolysis
  - S-stereochemistry. The R group interacts with residues in the active site via hydrogen bonding



When target based screening goes wrong
- Target based screening assumes that the cause of disease is known
- Since 1901, the cause of Alzheimer's disease was believed to be beta-amyloid plaques in the brain
- Hundreds of drug trials based on this mechanism have failed to treat the progression of the disease
- PBT2 is a compound developed by Prana, a Melbourne-based pharmaceutical company
- Trials in 2007 showed that it reduced concentrations of beta-amyloid
- In 2014, the results of clinical trials in Alzheimer's and Huntington's disease were announced

Screening methods
- Several types of approaches to screening: random screening, focused screening and high-throughput screening
- Random screening
  - Absence of known drugs/compounds with desired activity
  - Fixed therapeutics objectives at the onset of the experiments
  - All compounds are tested in the bioassay without regards to structure

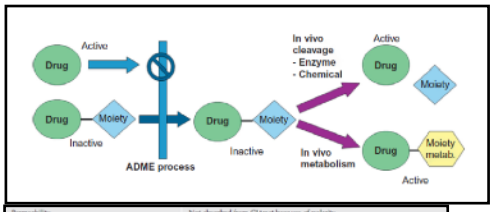
- Can be applied to new chemical entities - arising from chemists through synthesis, or natural products by isolation/purification
- Historically, random phenotype screen is better at identifying first in class drugs

Focused screening
- Also known as non-random targeted screening
- Vague resemblance to weakly active compounds - from random screens
- Has a better hit rate than random screening

High-throughput screening (HTS)
- Large numbers of compounds tested across a large number of biological targets
- Advancement in robotics and miniaturisation of in vitro testing
- Single compounds and mixtures can be screened
- Combinatorial chemistry can also be used to simultaneously generate thousands of compounds for screening
- Compound libraries are very valuable to companies

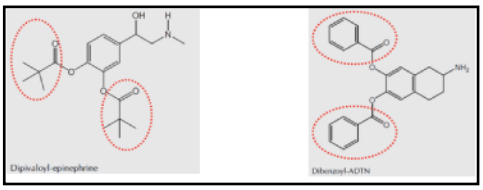
Prodrugs (L3)

Defintion
- A structural modification of an active drug that is designed to improve a drug property that limits pharmacokinetics and target exposure, and then is converted (activated) in vivo to release the modification and produce the active drug
- Utility of Prodrugs:
  - Permeability, Solubility, Metabolism, Stability, Transporter, Safety, Pharmaceutics

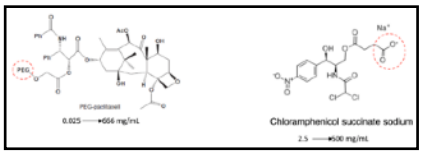


Prodrugs Strategies

Increase Permeability
- Most commonly used to increase permeability of compounds by masking the polar functional groups and hydrogen bonds with ester or amide linkers to increase lipophilicity
- An ideal ester/amide prodrug should exhibit:
  - High passive permeability
  - Resistance to hydrolysis during absorption
  - Hydrolyse to parent (Active) drug rapidly and quantitatively after absorption

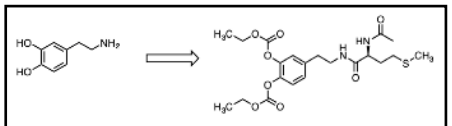


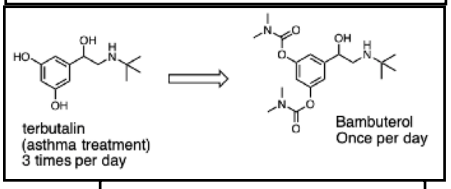
Increase Solubility
- Commercial prodrugs with greater solubility than the active drug
- Prodrugs with a **non-ionisable promoieties** (e.g. glycol, polethylene glycol (PEG), sugars) can typically improve solubility by 2- to 3- fold
- Prodrugs with **ionisable promoieties** (e.g. phosphate) can increase solubility by orders of magnitude

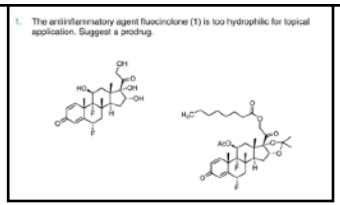


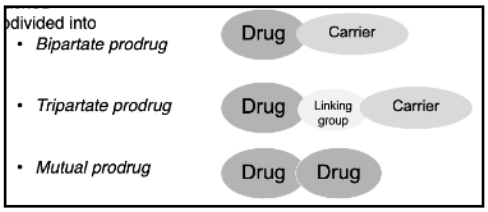
Reduce Metabolism
- Prolong the half-life of the parent drug by masking labile functional groups
- Essentially slow-release drugs
  - Dopamine is not orally available, due to rapid metabolism
  - Docarpamine is an orally active dopamine prodrug
  - The bisethylcarbonates are hydrolysed in the intestine and the amide is converted in the liver

  - Bambuterol is a dicarbamate prodrug of terbutalin
  - The phenolic groups are protected from metabolism









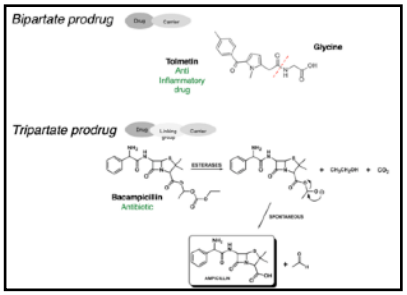
- The carbamates are slowly hydrolysed to release the parent terbutalin
- The slow metabolism results in a longer half-life

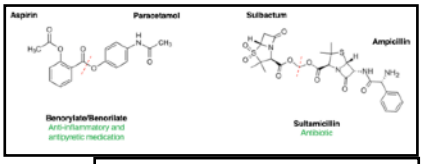
Types of Prodrugs

- Two classes of prodrugs: **carrier-linked prodrugs and bioprecursors**

Carrier-linked prodrugs

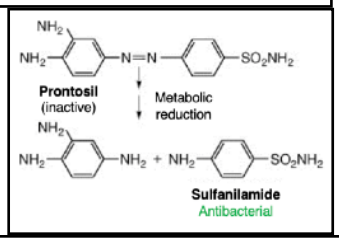
- Compound that contains an active drug linked to a carrier group that can be removed enzymatically
- Bond — must be labile — removed in vivo
- Carrier group — must be non-toxic and biologically inactive when detached
- Subdivided into
  - Bipartate prodrug: Drug + carrier
  - Tripartate prodrug: Drug + linking group + carrier
  - Mutual prodrug: Drug + Drug





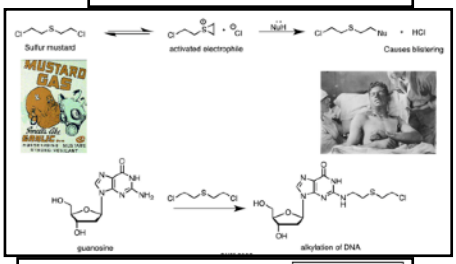
Mutual prodrug

- Consist of two pharmacologically active agents coupled together — act as a promoiety for the other agents and vice versa

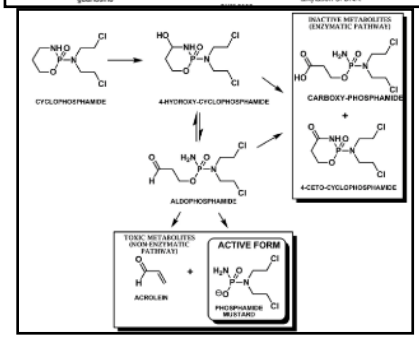


Bioprecursor prodrugs

- Produce their effect after in vivo chemical modifications (pharmacokinetics lecture) of their active form
- Rely on **oxidative or reductive activation reactions** - produce new compounds that may be active or further metabolised to an active metabolite



Bioprecursor prodrugs - sulfur mustards (chemical weapons)



Bioprecursor prodrugs - nitrogen mustard drugs:

- Precursor drug is inactive (deactivation by phosphamide), allowing oral dosing
- Inactive form is produced in cells with high levels of aldehyde dehydrogenase enzyme (bone marrow, liver and intestines) limiting its toxicity

Pharmacokinetics 1 (W2)

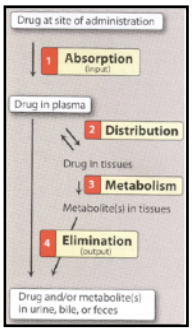
Liberation and Absorption (L1)

Pharmacology

- Pharmacokinetics
  - What does the body do to the drug?
- Pharmacodynamics
  - What does a drug to the body

Pharmacokinetics

- (L) A D M E
- Liberation — release of the drug from the formulation
- Absorption — process of entering blood circulation
- Distribution — transport of compounds from the point of administration or absorption to its site of action
- Metabolism — transformation of compounds into daughter metabolites
- Excretion — elimination of substances from the body (vs. accumulation)



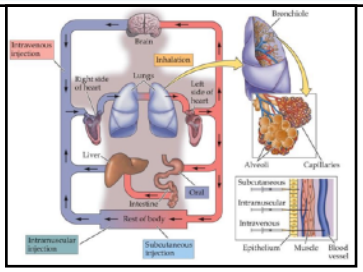
Excipients and Liberation

- Example: Oral Administration — Tablet
- 5-10% **active drug**
- Other % are **excipients** - fillers, binders, disintegrants, lubricants, colouring agents and preservatives

Excipient	Function
Cellulose	Adsorbent and suspending agent
Corn starch	Binder
Gelatine	Suspending agent, tablet binder and viscosity-increasing agent
Hydroxy propyl methyl cellulose	Coating agent, film-former, rate-controlling polymer for sustained release, stabilizing agent, suspending agent, tablet binder, viscosity-increasing agent
Ferric Oxide	Colour
Magnesium stearate	Lubricant
Titanium dioxide	Coating agent and opacifier
Sucrose	Suspending agent, sweetening agent, viscosity-increasing agent

Drug Administrations

- Different administration affects
  - Kinetics, interactions, uptake, metabolism, excretion, etc
- Drug can influence appropriate administration method
  - Lipophilic vs hydrophilic substances, MW, pH, etc
- Intravenous injection, intramuscular injection, subcutaneous injection, inhalation, oral,



Oral Administration

- Safe, commonly used
- Delay before uptake into the blood stream
- Drug can be metabolised before absorption
- Convenient and economical
- Food can affect absorption
- Limited absorption for some drugs

Intravenous administration:

- Straight into the blood stream - absorption not required
- Often immediate effects, good in emergencies
- Tittration possible
- Large volumes possible
- Expensive
- Not suitable for some oily/poorly absorbed substances
- Adverse effects possible
- Aseptic techniques required

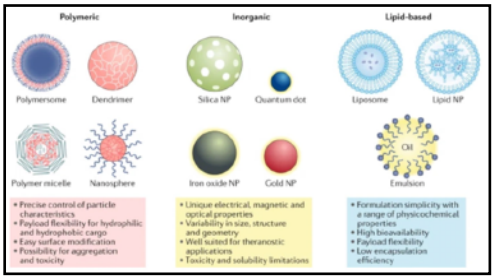
Other methods of administration

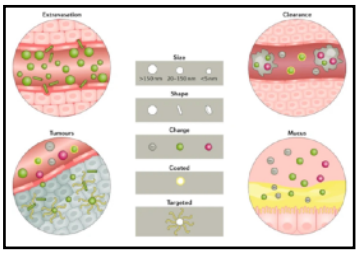
- Epicutaneous/topical
- Transdermal (patch)
- Subcutaneous
- Nasal
- Intramuscular
- Intrathecal (spine)
- Intraarterial
- Rectal

- Sublingual
- Inhalation, etc

Current challenge is achieving small needle size (<100 micrometer) with biocompatible material (e.g. polyactic acid)

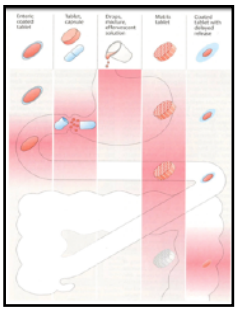
Nanoparticles — an emerging strategy for drug delivery
- Polymeric
  - Precise control of particle characteristics
  - Payload flexibility for hydrophilic and hydrophobic cargo
  - Easy surface modification
  - Possibility for aggregation and toxicity
- Inorganic
  - Unique electrical, magnetic and optical properties
  - Variability in size, structure and geometry
  - Well suited for theranostic applications
  - Toxicity and solubility limitations
- Lipid-based
  - Formulation simplicity with a range of physicochemical properties
  - High bioavailability
  - Payload flexibility
  - Low encapsulation efficiency



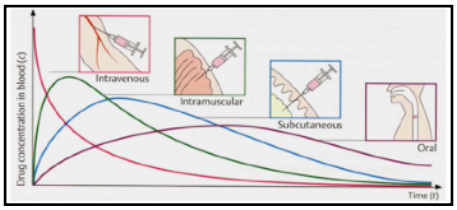


Nanoparticle distribution and clearance
- Size, shape and charge affect the distribution and clearance of nanoparticles

Drug release from nanoparticles
- Diffusion
- Solvent
- Chemical reactions
  - pH, hydrolysis, enzymes
- Stimuli-sensitive
  - Magnetism, light, ionic strength (Na+, Cl-, Ca2+), heat, ultrasound



Liberation
- **Liberation**: release of the drug from the formulation
- GI tract: different pH, enzymes, different digestive functions
- Stomach, pH ca. 1-3M, HCl
- Small intestine (digestion, enzymes): Duodenum with pH 6, jejunum (7, up to 9), ileum, pH 7.4
- Large intestine (abs. Nutrients, H2O):
  - Cecum (with appendix), pH 5.7
  - Colon, rectum, pH 6.7



Absorption
- **Absorption**: Process of entering blood stream, uptake of drugs
- Important for drug development/medicinal chemistry since the drug **must be absorbed** before an **effect** can take place
- Pharmacokinetics profile can be altered: changing the formulation, changes on the molecular level = affect absorption
- Stomach wall is NOT designed for significant uptake of its content
- A modest proportion of acetylsalicylic acid (aspirin) is absorbed across the stomach wall in neutral form (I.e. **protonated**)
- Amines are often **protonated** in the stomach and cannot undergo gastric absorption