Preformulation

Preformulation involves the **characterization** auf a drug's **physical, chemical** and **mechanical** properties to choose what other ingredients (= **excipients**) should be used in the preparation + to develop a **stable**, safe and **effective dosage form**. The important aspect is to understand the **solution behavior** of a given protein under a variety of stress conditions: freeze/thaw (*tauen*), temperature, shear stress (*Schubstress*) among others to identify mechanisms of degradation (*Verschlechterung*) and its mitigation (*Abschwächung*). Formulation is the **final medicine**.

Preformulation is a phase of the research and development process (R&D): \rightarrow converting **idea** into **candidate drugs** for development

Product development:

→ converting **candidate drugs** into products for registration and **sale**

→ <u>Hurdles</u>

Research	\rightarrow new compound (patentable?)	
	\rightarrow new biological mechanism (patentable?)	
	\rightarrow unmet medical needs	
	\rightarrow potent: a small dose works	
	\rightarrow selective: no side effects (is very unrealistic)	
Safety	\rightarrow despite wrong application no bad effects	
	\rightarrow non-toxic (-carcinogenic, teratogenic, mutagenic etc.)	
Clinical	\rightarrow tolerable side-effects profile e.g. in cancer therapy	
	\rightarrow efficacious (<i>wirksam</i>)	
	\rightarrow acceptable duration of action: How long should it work?	
Drug process	\rightarrow bulk drug can be synthesized/scaled up	
Pharmaceutical	\rightarrow acceptable formulation/pack: capsules, tablets,	
	\rightarrow drug delivery / product performance acceptable: drug has to be	
	released at a certain intestine	
	\rightarrow acceptable shelf-life	
	\rightarrow clinical trial process robust, can be scaled up	
Regulatory	\rightarrow quality of data, documentation of development process (AGES)	
Manufacturing	\rightarrow manufacturable	
	\rightarrow able to pass pre-approval inspection	
Marketing /	→ competitive	
commercial	\rightarrow meets customers' needs	
	\rightarrow value for money	
	\rightarrow commercial return	

Generica are reformulated, "old" products, mostly produced in India and Asia due to the fact that it's cheaper there.

- \rightarrow less hurdles
- \rightarrow only certain parts of the investigations have to be shown (= **bio-waiver**)
- \rightarrow lower profit

To generate cash flow, pharmaceutical companies must ensure that new discoveries are frequently brought to the market \rightarrow also to fund the next generation of compounds. This cycle of events is called the **product life cycle**. Costs: 500 Mio. – 2 Bn. US\$ which include failures during development.



Strategic Research

The strategic research of a particular company is usually guided by factors such as its inherent research competence and expertise, therapeutic areas of unmet medical need and market potential/commercial viability. Companies often wish to **develop a portfolio of products** within a **special therapeutic are** to capture a segment of the market. By focusing on a particular therapeutic area, a company can build on its existing expertise and competence in all of its functions with the aim of becoming a leading company in that field.

Exploratory Research

Investigation of the biological mechanism and identification of a "**chemical lead**" that interferes with it. During this stage, **compounds are screened** for the desired biological activity. The aim is to find a chemical of molecular entity that interferes with the process and to provide a valuable probe of the underlying therapeutic problem.

Candidate Drug Selection

The chemical lead is used to generate specific chemical compounds with the optimal desired characteristics: potency, specificity, duration, safety, pharmaceutical aspects. During this stage, the chemical lead is optimized by testing a range of selected compounds in vitro and in vivo (animal) studies.

Exploratory Development

The aim is, to gauge how the **drug is absorbed** and **metabolized** in **healthy human volunteers**. Further small-scale studies are necessary to make a decision whether to progress the drug into full development or not = **Phase I clinical studies**.

Full Development

Phase II and III: patients suffering from the disease \rightarrow *several hundreds of patients take the drug to evaluate the effectiveness* + *side effects.*

Preferred drug synthesis and pharmaceutical properties for compounds **intended for oral solid dosage form** development:

Drug Synthesis Factors	Formulation/Drug Delivery Factors
least complex structure (no chiral centers)	exists as a stable, polymorphic form
only a few synthesis steps	non-hygroscopic
high yield (= Ertrag)	crystalline
non-explosive route, no safety issues	solid-state stability
commercial availability	oral <u>bioavailability</u> (tablet should solve)
low cost of goods compared to overall cost	not highly colored, no strong odor, no bad
of product on the market	taste
no predicted problems in scale-up e.g.	compatible with key excipients
performable synthesis	



McKinsey&Company assessed that a product that is **6 months late** to market will miss out on one third of the potential profit.

- \rightarrow only 3/10 are likely to achieve a fair return on investment
- \rightarrow fast development essential for profit and reinvestments in research and development
- \rightarrow keep it simple: balance between high quality and fast development

New chemical entities (NCE) = getting new registration for medicine: only ~ 22 per year. Costs up to 2 Bio. \$.

Many common diseases are well investigated and treatable leaving **no easy targets** \rightarrow trend goes to more complex molecules of large molecular weight (peptides, proteins, enzymes).

→ 7 stages of product development

Stage 1: Lead finding 1-2 years

- \rightarrow identification of biological **mechanism** and **chemical leads** that interfere with it
- → pre-knowledge on structure of therapeutic target makes it easier
- \rightarrow no information: try & error with cell cultures
- \rightarrow research & development

Stage 2: Candidate Drug Screening 1-2 years

 \rightarrow Structure/activity correlations

- \rightarrow Drug design
- \rightarrow laboratory scale preparation \rightarrow only a few mg are produced
- → HTS (high-throughput screening) + basic pharmacological/biochemical screening
- \rightarrow Preformulation

Stage 3: Candidate Drug Selection 1-2 years

- \rightarrow simple structure, not toxic
- \rightarrow fewer synthesis steps
- → passes carcinogenic, mutagenicity, LD50 tests
- → non-hygroscopic
- \rightarrow chemical stability, stable solid state
- \rightarrow bioavailability
- \rightarrow no strong colors, odors, tastes
- \rightarrow compatible with standard excipients (= Arzneiträgerstoff)
- \rightarrow 90% survival rate (shelf-life)

Stage 4: Preclinical studies 1-2 years, up to 6 years

- → testing on **non-human subjects** (animals allowed)
- \rightarrow dosage unrestricted

Stage 5: Phase 0, Phase I – Clinical studies

 \rightarrow Phase 0: pharmacodynamics and pharmacokinetics, bioavailability, half-life (rough screening)

→ Phase I: testing on **healthy volunteers** for dose-ranging, might be risky

Stage 6: Phase II and III, Studies + Launch 4-6 years

- \rightarrow Phase II: testing on **patients**, assess efficiency and safety
- \rightarrow Phase III: thousands of patients treated (most expensive)

Stage 7: Phase IV and V

- \rightarrow Phase IV: Postmarket surveillance (3-5 years): troubles have to be figured out
- \rightarrow Phase V: research on data collected

Three main categories of active pharmaceutical ingredients = APIs

- 1. Small molecules (caffeine, ibuprofen)
- 2. Large molecules (insulin)
- 3. Botanical extracts (digitalis for heart diseases)



Balance of forces determines <mark>solid state</mark> and <mark>solution</mark> properties: electrostatic, Van der Waal, solvophobicity and H-bonding

Molecule Properties:

Why is it important?

 \rightarrow Cell interaction

→ Membrane transport: diffusion, facilitated diffusion (channels), osmosis, active transport (sodium Na out, potassium K in the cell), exocytosis: exportation in vesicles, endocytosis: membrane flips in and builds a vesicle

 \rightarrow Protein binding: a protein that causes diseases

could be stopped by changing its movement, **artificial proteins** produced to see what happens when the drug interacts

→ Drug formulation: knowledge und interactions for correct form of drug

Important properties are dissociation, partitioning (Abtrennung) and solubility.



→ Dissociation

Many candidate drugs are weak acids or bases. One of the most pertinent determinations prior to development is the **pKa** or ionization constant.

$$\mathrm{pH} = -\log_{10}(a_{\mathrm{H}^+}) = \log_{10}\left(\frac{1}{a_{\mathrm{H}^+}}\right) \;\cong \text{-log}\left(\mathsf{C}_{\mathsf{H3O+}}\right)$$

pH = negative decimal logarithm of the hydrogen ion activity in a solution

acid dissociation constant (pKa) = $-\log_{10}(K_A)$ pKa < 0 very strong acid (HClO₄, -10) pKa = 0 - 4,5 strong acid (HF, 3,18) pKa = 4,5 - 9,5 weak acid

dependency of pH and pKa: cHA= molare concentration of the undissociated weak acid cA- = molare concentration of the conjugate base

$$pH = pKa + \log \frac{c_A}{c_{HA}}$$

basidic dissociation constant (pKb) = pKa+pKb=14

	100	рΗ	рКа	Ionization
ionization %	100	0	4	99.99
How much is ionized at:	$1 + 10^{pH - pKa}$	1	4	99.90
		2	4	99.00
$r K_{0} > r H > 500/$		3	4	90.90
$pKa > pH \rightarrow > 50\%$		4	4	50.00
$pKa = pH \rightarrow = 50\%$		5	4	9.09
$pKa < pH \rightarrow < 50\%$		6	4	0.99
		/	4	0.09
		0	4	0.001
mothods for determination of nKa		10	4	1F-04
methods for determination of pixa.		11	4	1E-05
\rightarrow potentiometric titration		12	4	1E-06
\rightarrow UV spectroscopy		13	4	1E-07
\rightarrow solubility measurements		14	4	1E-08
\rightarrow HPLC	14.			
\rightarrow capillary zone electrophoresis	14			
	12			A REAL PROPERTY AND A REAL
\rightarrow foaming activity				f l
or ACDpKa – software, SPARC (25°C)	10			
				This represents the equivalence point in
Molecules may have multiple dissociat	$\frac{1}{100}$ \pm 8			the titration.
interesting have multiple dissociat	~ ~ ~			
centers:	0		j	
$pKa_1 = 1.27$	4		and the second s	
$pKa_2 = 4.27$	4		T	his is halfway to the
Good buffer between $nH-1.3 - 4.4$	2	And the state of t	equival	lence point where the pH
Good build between pii=1.5 +.+			= pK _A o	buffering region.
	0 +			
	0 5 10) 15 20	25 3	30 35 40 45





To provide high bioavailability for therapeutic action, most often individual molecules are required.

like dissolves like

- Electrolytes dissolve in conducting solvents.
- Solutes containing hydrogen are capable of forming hydrogen bonds typically dissolve in solvents capable of accepting hydrogen bonds and vice versa.
- Solutes having significant dipole moments dissolve in solvents having significant dipole moments.
- Solutes with low or zero dipole moments dissolve in solvents with low or zero dipole moments.

Solubility (and solutions) provide information on **how many solvent molecules** are there/required in respect with the solute.

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble or insoluble	10,000 and over

can be expressed:

- \rightarrow g (solute) / kg (solvent)
- \rightarrow molarity (mol/V)
- \rightarrow molality (mol/kg)
- \rightarrow weight %

Class of solvents:

1. Proctic solvents: water, methanol, formamide (hydrogen bond donors)

2. <u>Bipolar aprotic solvents</u>: DMSO, acetonitrile, THF (no hydrogen bonds but dielectric constants is greater than 15)

3. Aprotic solvents: pentane, benzene (dielectric constant is weak, non-polar solvent)

Prediction of good solvent quality:

 \rightarrow Hildebrand solubility parameter (HSP) is **not accurate**

→ Hansen solubility parameters: 3 dimensional

$(Ra)^2 = 4(\delta_{d2} - \delta_{d1})^2$	$+1(\delta_{p2}-\delta_{p1})^2$	$+ (\delta_{h2} - \delta_{h1})^2$
The energy from dispersion forces (van der Waals)	from dipolar intermolecular force	energy from hydrogen bonds

 $RED = Ra/R_0$

Parameters effecting solubility:

• <u>molecular size and substituent groups</u> Solubility debends on the number of solvent molecules that can pack around the solute molecule R_o, indicates the maximum difference in affinity tolerable for a "good" interaction to take place

RED < 1 the molecules are alike and will dissolve RED = 1 the system will partially dissolve RED > 1 the system will not dissolve

- <u>degree of ionization</u> amphoteric molecules, are materials that can act as base or acid depending on the environment ; isoelectric point (pI)
- <u>ionic strength</u>
- •
- <u>temperature</u>:

°t \uparrow = solubility \uparrow (endothermic process), exceptions for Na₂SO₄, Ce₂(SO₄)₃ **cloud point formation**: at certain t° break down of solvation forces, miscibility gap (Mischungslücke) higher t° redissolves molecule, turbiness (Trübe)

• <u>salt form</u>:

increase (salt in) or decrease (salt out) of solubility The effect of the additive depends on the influence it has on the structure of the water Setschenow equation

- pressure
- crystal properties
- <u>complexation</u>

The isoelectric point (pI) is the pH at which a particular molecule carries no electrical charge.



Important solvents for pharmaceutical application:

water, buffers (various pH), polyethylene glycol, propylene glycol, glycerin, sorbitol, alcohols, tweens and polysorbate, natural oils, organic solvents

Solubility Modulation:

- List of solvents
- Solubilizer
- Micelles
- Liposomes
- Nano particles
- Nanocrystals
- Encompass drug molecule, ... umschließen
- Huge surface, small particles have different floating behaviour (e.g. not effected by gravity, ...), ...

Saturation is the point at which a solution of a substance can dissolve no more of that substance and additional amounts of it will appear as a precipitate (Fällung). The point of **maximum concentration** (saturation point) depends on the **temperature** of the liquid and the **chemical nature** of the substances involved.

If conditions change (e.g. cooling) and the concentration is higher than the saturation point, the solution has become **supersaturated.**

Problems measuring solubility:

 \rightarrow samples containing other components: check for overlap in absorption spectra (maybe HPLC)

 \rightarrow samples containing particles: light scattering may result in wrong measurements (HPLC of filter)

Measurement methods:

- <u>Plate method for solubility</u>: saturated solution is filtered with a **filter plate** and supernatant (Überstand) is measured by **UV spectroscopy** (multiple wavelength) → example with DMSO
- <u>UV-VIS adsorption spectroscopy:</u> adsorption of UV and visible light (VIS).

→ Spectral range: correlation between wavelength of the absorbed radiation and observed color (200-800nm)



→ <u>Absorption edges:</u> which bonding absorbs in which region? In the lab are 200-1800nm possible.

Example: β -Carotene, $\lambda_{max} = 460$ nm = orange

• <u>Beer-Lambert Law:</u> The Absorbance is equal to the log of ratio between the incident beam intensity and the exciting beam intensity.



Is the concentration very high, absorption is **not linear** due to multiple scattering. Furthermore, a change in environment causes change in the absorbance bands \rightarrow always measure as many wavelengths as possible.

→ Partitioning

Lipophilicity:

Measure of how compatible molecules are with apolar solvents, oils, waxes, ...

-> important for drug-cell interaction

Partition coefficient (log P):

Number of polar molecules (soluble in aquaeous phase = hydrophilic) vs. number of apolar molecules (soluble in apolar phase = lipophilic)

A polar and an apolar phase are needed. Most of the time **Octanol** and **Water** are mixed and shaken (in separation funnel). N-Octanol properties are thought to be similar to lipid bilayer membranes and simulates (to certain extent) ability to passively **diffuse across biological membranes**.

Drug permeability in brain capillaries (y-axis) as a function of partition coefficient (x-axis).[[]



Also, **Cyclohexane/water** are used. Unlike noctanol, cyclohexane has **no H-bonding characteristics** \rightarrow similar to blood-brain barrier.

 $\log P = \frac{[\text{unionized compound}]_{org}}{[\text{unionized compound}]_{aq}}$

Partition coefficient (log P):

= 0	compound is equally soluble in water and the partitioning solvent
= 5	compound is 100.000 times more soluble in partitioning solvent
= - 2	compound is 100 times more soluble in water (= hydrophilic character)

log P (octanol/water) – log P (alkane/water) has been suggested to reflect hydrogen bonding capacity, which has implications for skin penetration. Compounds with high log P values and low H-bonding capacity can get past **ester/phosphate groups** in skin membranes.

→ <u>The distribution factor</u>:

What happens, if some molecules are dissociated?

$$\log D_{
m oct/wat} = \log \left(rac{[
m solute]_{octanol}^{
m ionized} + [
m solute]_{octanol}^{
m un-ionized}}{[
m solute]_{
m water}^{
m ionized} + [
m solute]_{
m water}^{
m un-ionized}}
ight)$$

If no dissociation in the organic phase occurs it is simplified to the log D:

$$D = \frac{[\text{HA}]_{org}}{[\text{HA}]_{aq} + [\text{A}^-]_{aq}}$$

The log D correlates to the pH, pKa and log P as:

 $\log D = \log P - \log[1 + 10^{pH - pKa}]$

→ Various factors are measured automatically. An example is the Sirius T3. Further measurement methods are:

HPLC

 \rightarrow correlation retention time with similar compounds with known log P values

 \rightarrow Drawback: chemical structure must be known beforehand. Different chemicals may have different regression parameters.

<u>Membrane – permeability</u> with oil/water partition coefficient (see picture below)

<u>Solubility method for pKa</u>: useful for substances with **low solubility**. An aqueous solution is titrated until the free base/acid will precipitate. pKa can be calculated from the solubility product \rightarrow not precise.

Shake-Flask method for logP/D: Photometric measurement (UV/VIS) of an aqueous solution from a shake-flask (Schüttelmethode) \rightarrow takes 3 days for equilibrium as well as pre-saturation.



Methods without experiments:

Not necessarily linear behavior!

Atomic based prediction: with PubChem Fragment based prediction: = group contribution; log P \rightarrow electronic and steric effects Data mining prediction: by using the internet (large data sets) Molecule mining prediction: similarity matrix based prediction, automatic fragmentation

scheme into molecular substructures

Quantitative structure – activity relationships (QSAR)

 \rightarrow relate measurements on a set of "predictor" variables to the behavior of the response variable. **Chemical and biological activity** of the substances:

- → Hammet parameter: correlation between **electronic** properties of organic acids and bases with their **equilibrium constants** and reactivity
- → Hansch analysis: relationship between **lipophilicity** and **biological activity**

Lipinsky's Rule of 5 for a high oral bioavailability:

- Not more than **5 hydrogen bond donors** (N, O atoms with one or more H atoms)
- Not more than **10 hydrogen bond acceptors** (N, O)
- \circ Molecular weight < 500 D
- Octanol-water $\log P < 5$ (portioning coefficient)

Extension of these rules by Ghose

- $\circ \log P 0.4 5.6$
- molar refractivity 40 130 (measures the total polarizability of a mol of a substance, depends on temperature, index of refraction and pressure.
 It is defined as: Molecular weight 160 480 D, number of atoms 20 70

Relation between biological activity and log P (Optimum)

central nervous system penetration	2-2.7
Oral absorption	1.8
Sublingual absorption	5.5
Intestinal absorption	1.35
Colonic absorption	1.32
Percutaneous	2.6 + low molecular weight

Formulation decision – design of drug formulation

low log P (< 0)	injectable
medium (0-3)	oral
high (3-4)	transdermal
very high (4-7)	toxic: fatty tissues

RULES are a good estimation but may be influenced and not that accurate

→ Solution properties

Viscosity of solution depends on solvent, solutes, temperature, ...

- \rightarrow molecules in a fluid/solution show a relative motion to each other
- \rightarrow internal friction counteracts motion
- \rightarrow moving plate "feels resistant force" (**F**)

 \rightarrow depends on Area (A) and speed (v) and distance of plates



Dependency of concentration of polymer/macromolecule in solution on the viscosity:

- → increase of **polymers** = increase of concentration = increase of viscosity
- → dilute solution: individual polymer coils in solution; almost no interaction
- → semi-dilute solution: polymer coils start to strongly interact.



There are three cases of velocity dependent fluid behavior:

- <u>Newtonian</u>: The quicker you move the liquid, the more force you need
- <u>Shear thinning</u>: Viscosity drops by pushing velocity it faster: in **polymer solutions** and **macromolecule solutions**

The breakdown of interaction forces the polymer coils to rearrange.

• <u>Shear thickening</u>: Viscosity increases by higher velocity. It is often observed in **colloidal systems** (starch = Speisestärke in water)

The change of viscosity properties over time + recovery of initial properties after certain time is named **Thixotropic properties/effect**.

Reason: breakdown of interaction. Polymer coils rearrange which leads to equilibrium condition after some **relaxation time**. Example: shaking a bottle of ketchup.

Viscosity measurements: Ball settling, Rheometer (used by Danone for their puddings)

Solvent quality of polymers and macromolecules

 \rightarrow <u>poor solvent quality</u>: coil tries to minimize contact with solvent which leads to a **compact structure** (small radius)

 \rightarrow <u>theta solvent quality</u>: no solvent – polymer interaction forces, coil confinement as in **vacuum**: bigger structure

 \rightarrow <u>good solvent quality</u>: Polymer – solvent interaction fabored over polymer – polymer interaction - **expanded polymer coil structure**

Polymers can dissolve under **all conditions** but due to the large number of configurations dissolving these processes may take very long \rightarrow up to a few weeks.

<u>Determination:</u> Small angle neutron scattering, X-Ray scattering, light scattering, rheology, ...

Surface tension = work required to increase the surface area

$$\varepsilon = \frac{\Delta W}{\Delta A}; \quad [\varepsilon] = \frac{J}{m^2}$$

An addition of **amphililic** molecules results in **reduction** of surface tension:

 \rightarrow surfactant molecules

 \rightarrow if surface is hosted with molecules, the tension is reduced

 \rightarrow molecules start to form **micelles** if the amount on the surface is saturated

→ drug – solvent interaction strongly changed: might enable specific **drug delivery route**

In case of wanting to express a certain surface tension, the "cmc" numbers have to be considered. force is not 0 -> surface tension

$$\vec{F}_{R} \neq 0$$

Abb. 6.29. Resultierende Anziehungskraft aller anderen Moleküle auf ein Molekül im Inneren und an der Oberfläche einer Flüssigkeit

molecules floating around inside the vessel



water (very polar solvent)

Ober flächenspannung







log Tensidkonzentration

Chemical Kinetics and Stability

The purpose of stability testing is to provide evidence on how the **quality** of a drug substance/product **varies** with time under the influence of a variety of **environmental factors** such as temperature, humidity and light and to establish a retest period for the drug substance of a **shelf life** for the drug product and recommended storage conditions.

Products = aA + bB + ...The **rate of reaction** is:

Rate
$$= \frac{1}{a} \frac{d[A]}{dt} = \frac{1}{b} \frac{d[B]}{dt} = \cdots k[A]^a [B]^b \cdots$$
 k = rate constant

Half life is the time required for one-half of the material to disappear.

Shelf life is the time required for 10% of the material to disappear (0.9 A left).

Expiration date is the date placed on the container label of a drug product designating the time prior to which a batch of the product is expected to remain within the approved shelf-life specification if stored under defined conditions after which it must not be used.

Which factors affect reaction velocity?

- \rightarrow concentration
- \rightarrow temperature

 \rightarrow solvents: reactions of non-electrolytes is related to **internal pressures** or **solubility parameters, ionic strength, dielectric constant**

- \rightarrow catalysts
- \rightarrow light

Stability of pharmaceuticals

Pharmaceutical decomposition can be classified as hydrolysis, oxidation, isomerization, epimerization, and photolysis, and these processes may affect the stability of drugs in liquid, solid, and semisolid products. Mollica et al.³⁰ reviewed the many effects that the ingredients of dosage forms and environmental factors may have on the chemical and physical stability of pharmaceutical preparations.

Photodegradation (e.g. UV spectroscopy)

Light energy, like heat, may provide the activation necessary for a reaction to occur. Radiation of the proper frequency and of sufficient energy must be absorbed to activate the molecules. The energy unit of radiation is known as the *photon* and is equivalent to one *quantum* of energy. Photochemical reactions do not depend on temperature for activation of the molecules; therefore, the rate of activation in such reactions is independent of temperature. After a molecule has absorbed a quantum of radiant energy, however, it may collide with other molecules, raising their kinetic energy, and the temperature of the system will therefore increase. The initial photochemical reaction may often be followed by thermal reactions.

Stress testing

It is used to elucidate the **intrinsic stability** of the drug substance. It is part of the development strategy. It includes

- \rightarrow effects of temperature in 10°C increments
- \rightarrow humidity
- \rightarrow oxidation
- \rightarrow photolysis

Dissolution vs. Release



<u>Membrane transporters</u> facilitate drug transport through membranes (= channels). In the end, drugs always reach a blood vessel.

<u>Osmosis</u> = transport of a solvent through a semipermeable membrane. The driving force is the **osmotic pressure**.

<u>Ultrafiltration</u> is used to separate **colloidal particles** and macromolecules. A membrane + hydraulic pressure is used to force the solvent through the membrane: large solute molecule can't pass the micropores \rightarrow used in **paper industry**, research to purify **albumin** and enzymes.

Microfiltration is a process with membranes of larger pore size \rightarrow to remove **bacteria** from injections, foods, water.

Dialysis separates solute and solvate as well but without the hydraulic pressure.

Driving forces:

 \rightarrow Concentration (passive diffusion, drug dissolution)

 \rightarrow Pressure (osmotic drug release)

 \rightarrow Temperature (Lyophilization = Gefriertrocknen for not destroying secondary structures),

mivrowave-assisted extraction

 \rightarrow Electrical potential

Diffusion



Diffusion across a thin film: from higher concentration to lower concentration until they are kept constant on both sides of the film. At steady sate, the concentrations remain constant at all points in the film; the c-profile inside is **linear**, the **flux** is **constant**.

Diffusion through membranes: Energy is needed to overcome barriers.

Fick's second law predicts how diffusion causes the concentration field to change with time.

$$\frac{dC(x,t)}{dt} = -\frac{dJ(x)}{dx} = +D\frac{d^{2}C(x)}{dx^{2}}$$

The rate of dissolution by Noyes and Whitney

$$\frac{dm}{dt} = \left(\frac{D \times A}{h}\right) \times \left(Cs - Ct\right)$$

Fick's first law is applicated here. m....amount of dissolved material A....surface area between dissolving substance + solvent

D....diffusion coefficient

h.... thickness of the boundary layer

Cs.... concentration of the substance on the surface

Ct....concentration of the substance in the bulk of the solvent

->The **intrinsic dissolution rate** is used for getting to know **A**. An amount of drug dissolved per unit time and unit surface area (tablet gets smaller during dissolution) \rightarrow dissolution getting slower

->The rotating disc method makes this process faster.

->Nano-sized particles dissolve faster since their surface area is bigger.

->Dissolution rate will provide estimate on **bioavailability**.

Dissolution testing:

→ USP apparatus 1: **Basket**, for capsules, granules, pellets, floating drugs which disintegrate

slowly



 \rightarrow USP apparatus 2: **Paddle**, for tablets and capsules

 \rightarrow USP apparatus 3 & BioDis for tablets, ...

 \rightarrow USP apparatus 4 with a flow through design = very handy: capsules, tablets, gels, cremes, patches

 \rightarrow USP apparatus 5 and 6: **Paddle over disc** for patch holder (hormones, nicotin) = rotating cylinder

The plot shows the dissolution profile of **Paracetamol** tablets.

Zero-order release = process of constant drug release (e.g. oral osmotic tablets) = same amount of drug release per unit of time:

 $Q = Q_0 + K_0 t$ (zero-order release constant)





Proposed limits of drug dissolution on solubility to avoid absorption problems:

Solubility in pH 1-7	>10mg/ml at all pH
Solubility in pH 1-8 and dose	complete close dissolved in 250ml in all pH
Water solubility	>0.1mg/ml
Dissolution rate in pH 1-7	>1mg/min/cm ² at all pH

→ Permeation

= penetration of permeates (solid, liquid, gas) through a solid.

Basic types of animal tissue are connective tissue, nervous tissue, muscle tissue and epithelium tissue (e.g. functions of epithelial cells: absorption, protection, transcellular transport, detection of sensation).

Drug transport across epithelial cell barriers is difficult to predict:

for example mucus layers try to prevent intake of unwanted substances = xenobiotic screen There is a large **interindividual variability** in the intestinal transport of drugs; different for each person + different **administration routes** (oral, sublingual, transdermal, ...) must overcome different barrier mechanisms for permeation.

Bioavailability = fraction of administered dose that reaches the systemic circulation and shows action

intravenously = 100% Bio-availability; via other routes bioavailability decreases

Bioavailability is one of the essential tools in pharmacokinetics, because it has to be considered when you calculate the dosage for non intravenous routes of administration. -> expressed with the letter F

How to test permeation?

Easiest approach is to administer at different routes and test if the substance reaches desired region (brain, blood)

 \rightarrow ONLY allowed after stage 4 + ethical commissions have to agree

→ best scenario: to find in-vitro or ex-vivo experimental setups

Apply drug substance of formulation on one side of a barrier (membrane out of polymer, glass fiber, cellulose, ..., cell culture) and test how much is able to diffuse to the other side. Unfortunately, bad correlations occur with physiological mechanisms. Model systems with good in vitro – in vivo correlations are required.



→Permeability Assays:

Multi-screen acceptor plate can be used to capture passively transported compounds (e.g. 96-well filter plate). Advantages:

- \rightarrow simultaneous testing of solutions and formulations possible
- \rightarrow various measurements with same solution and formulation
- \rightarrow statistical evaluation possible
 - <u>Parallel Artificial Membrane Permeability Assay (PAMPA):</u> The multi-screen-IP plate has a hydrophobic PVDF membrane which supports a lipid bilayer for PAMPA assays. (details in script)
 - 2. Caco-2 Assays:

= continuous line of heterogeneous human epithelial colorectal adeno carcinoma cells. They become differentiated under specific conditions and functionally resemble their phenotype. They express tight junctions, enzymes and transporters.

3. <u>Ex-vivo Assays</u>:

A **diffusion** of a drug is measured: through skin section, intestinal section etc., which is placed between two open vessels

 \rightarrow Franz Cell Apparatus: measured how well the drug molecule penetrates the cells (HPLC), but hard to prepare, IVIVC not good, sample degrades fast without working metabolism

 \rightarrow Ussing chamber: same procedure, works better than FC apparatus

4. In-vivo Assays:

An ethic commission must agree on animal testing. Standards: empty stomach, cross-over design, n > 6, at least 3 formulations in one study

5. <u>Ex-vivo model:</u>

= **Human placenta barrier** which prevents the baby from metabolizing toxic molecules.

Biopharmaceutical Classification System

BSC is guidance for **predicting** the intestinal drug absorption. It allows restricting the prediction using the parameters **solubility** (based on USP) and **intestinal permeability** (based on comparison to injections). (85% of drugs are administered orally)

Biopharmaceutical classification system.		
Class	Solubility	Permeability
I	High	High
П	Low	High
11	High	Low
IV	Low	Low

High solubility: Highest dose strength is soluble in < 250ml over a pH-range of 1-7.5 at 37°C **High permeability:** Absorption > 90% compared to intravenous dose

- I. High solubility, High permeability, Rapid dissolution for biowaiver, Route of elimination: metabolism or extensive, Transporter effects are minimal e.g. Diazepam, Buspirone, Lidocaine
- II. Low solubility, High permeability, Route of elimination: metabolism or extensive, efflux transporter effects are predominant e.g. Cisapride, Lovastatin, Tamoxifen
- High solubility, Low permeability, Renal and/or billary elimination of unchanged drugs, Metabolism is poor, Transporter: absorptive effects predominant e.g. Atropine, Lisinopril, Penicillins
- IV. Low solubility, Low permeability, Renal and/or billary elimination of unchanged drugs, Metabolism poor, Transporter: absorptive and efflux transporters can be predominant e.g. Collistin, Necmycin
- V.

In vitro – **in vivo correlation IVIVC** = predictive mathematical treatment describing the relationship between an in vitro property of a dosage form (rate or extent of drug release) and an in vivo response (drug concentration in plasma or absorption).

Level A: relation between in vitro solution and **absorption** in the body (most accurate) Level B: statistical analysis

Level C: correlation depends on single values of the in-vitro – in-vivo dependence like w% dissolved in 4h or c_{max}

Level D: combination of the above

IVIC is therapeutical meaningful, the release properties influence resorption

<mark>→ <u>Crystals</u></mark>

Solid state properties

Most medications are sold in a solid formulation. Solid state properties have impact on:

- \rightarrow processing and manufacturing
- \rightarrow dissolution properties: bioavailability
- \rightarrow stability and shelf life

Solids can be:

long range order in all 3 dimensions, direction dependent properties

long range order in 3, 2 or 1 dimension (directional order), direction dependent properties

no long range order, isotropic properties



Structure gives information on: mechanics (hard, elastic), electrical/optical/thermal properties, anisotropy (different directions possible), reactivity, solubility, dissolution rate, ... **Morphology** gives information on: size, shape, surface shape, roughness, ... **Structure and morphology** have effects on fabrication, process capability, properties \rightarrow application

The **crystal lattice** is a mathematical arrangement of points. A **unit cell** (smallest volume required to describe an entire crystal) is described with lattice constants (a, b, c) and lattice angles (α , β , γ). Any lattice point can simply be reproduced by a translation operation with lattice vectors = **periodicity**.

7 crystal systems are possible:

crystal system	lattice constants	angles
triclinic	$a \neq b \neq c \neq a$	$\alpha \neq \beta \neq \gamma \neq \alpha$
monoclinic	$a \neq b \neq c \neq a$	$\alpha = \gamma = 90^{\circ}; \beta \neq 90^{\circ}$
orthorombic	$a \neq b \neq c \neq a$	$\alpha = \beta = \gamma = 90^{\circ}$
tetragonal	$a = b \neq c$	$\alpha = \beta = \gamma = 90^{\circ}$
rhombohedral	a = b = c	$90^{\circ} \neq \alpha = \beta = \gamma < 120^{\circ}$
hexagonal	$a = b \neq c$	$\alpha = \beta = 90^{\circ}; \gamma = 120^{\circ}$
cubic	a = b = c	$\alpha=\beta=\gamma=90^\circ$

Additional symmetry operation allows **14 lattices** (=Bravais lattice) and **230 space groups** (crystallographic, Fedorov groups).

➔ Polymorphism

= a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of that compound in the solid state

- enantiotropic: one polymorph can be **reversibly changed** into another one by varying temperature of pressure
- monotropic: the change between two forms is **irreversible**

Depending on the interaction forces on the preparation route, one molecule might pack in various ways (e.g. Estron has 3 forms, Carbamazepine has 5 forms). One of the most often used example is **ROY** which has 10 polymorphs and consists of red, orange and yellow crystals.

Paracetamol has 3 forms whereas form 1 is not suitable for tablet production since proper shear planes are absent. Form 3 lacks on long time stability, so form 2 is typically used.

Pseudo-polymorphism = phenomenon whereby solvent or water is incorporated in the crystal lattice which leads to higher solubility and dissolution rates.

Hydrogen bonding networks lead to **coherence** (Zusammenhang) of the crystals. Three types of hydrates have been identified:

 \rightarrow Isolated lattice site water: water molecules are not in contact with each other

 \rightarrow Lattice channel water: molecules are hydrogen bonded and perform space-filling role

 \rightarrow Metal ion coordinated water: in the salt of **weak acids** (Ca²⁺), metal ion coordinates with water and is included in the growing lattice structure

Residual (verbleibende) solvents are classified as well:

 \rightarrow Class I: solvents to be <u>avoided</u> (carcinogens, environmental hazards e.g. benzene and carbon tetrachloride)

→ Class II: solvents to be <u>limited</u> (non-genotoxic carcinogens of irreversible toxicity, neurotoxicity, teratogenicity, reversible toxicity e.g. acetonitrile, toluene, methanol)

 \rightarrow Class III: <u>low toxic potential</u> with permissible daily exposures (PDEs) of > 50mg/d

(e.g. acetone, ethanol ethyl acetate, ethyl ether)

 Table 3 Physical properties that differ among the various crystalline forms of a drug substance¹⁷

1. Packing properties	a. Molar volume and density
	b. Refractive index
	c. Conductivity, electrical and thermal
	d. Hygroscopicity
2. Thermodynamic properties	a. Melting and sublimation temperatures
	b. Internal energy (i.e. structural energy)
	c. Enthalpy (i.e. heat content)
	d. Heat capacity
	e. Entropy
	f. Free energy and chemical potential
	g. Thermodynamic activity
	h. Vapor pressure
	i. Solubility
3. Spectroscopic properties	a. Electronic transitions (i.e. ultraviolet-visible absorption spectra)
	b. Vibrational transitions (i.e. infrared absorption spectra and Raman spectra)
	c. Rotational transitions (i.e. far infrared or microwave absorption spectra)
	d. Nuclear spin transitions (i.e. nuclear magnetic resonance spectra)

4. Kinetic properties	a. Dissolution rate	
	b. Rates of solid state reactions	
	c. Stability	
5. Surface properties	a. Surface free energy	
	b. Interfacial tensions	
	c. Habit (i.e. shape)	
6. Mechanical properties	a. Hardness	
	b. Tensile strength	
	c. Compactibility, tableting	
	d. Handling, flow, and blending	

→ Crystal growth

Protein solution \rightarrow supersaturation \rightarrow nucleation \rightarrow crystal growth \rightarrow 3D crystal

The liquid phase contains solute and solvent particles.				
Saturated solution:	$C = C_e$ (equilibrium concentration)			
Supersaturated solution:	$C > C_e$ (nuclei are formed)			
Undersaturated solution:	$C < C_e$			
->Crystal growth does not occur unless $C > C_e$				

Nucleation is a process in which the first tiny solid aggregates are formed. Two types of energy govern this process: attraction and surface Crystal growth is a **time**, **volume** and **concentration** (e.g. sodium acetate crystal) dependent process:

Common difficulties:

 \rightarrow supersaturation too high, too many tiny nuclei form

→ "bad" nuclei stimulate formation of amorphous precipitate = unwanted structure → supersaturated solutions with no spontaneous nucleation: crystal growth only when added to nuclei or "seeds".



Seeding

A seed provides a template on which further molecules can assemble (vereinigen). Energetically, it is more favorable to add to an already existing crystal plane than to create a new nucleus. Seeding allows controlling nucleation and some characteristics of the resulting crystal because it inherits some characteristics of the seed. Given proper **environment**, **time** and **patience**, the seed will enlarge into a crystal.

Heterogeneous nucleation

Disadvantages:

 \rightarrow Foreign solid substances promote surface where molecules can bind at a **lower degree of supersaturation**, which is a problem.

Siliconized glass surfaces build strong interactions with the crystal which breaks when attempted to dislodge.

Advantages:

 \rightarrow Adhesion to a regular surface may provide a suitable template to start an **ordered protein layer**.

Crystallizers are used in industry to achieve liquid-solid separation providing high purity products with a low energy input.

Conditions to screen for polymorphism/manufacturing:

- \rightarrow crystallization from different solvents, concentrations, speeds, temperatures
- \rightarrow crystallization from supercritical fluids
- \rightarrow precipitation
- \rightarrow evaporation
- \rightarrow grinding, compression, milling
- \rightarrow lyophilization
- \rightarrow spray drying (e.g. air brushing)

→ Crystal defects



- a) interstitial impurity atom loop
- d) vacancy (Lücke)

g) interstitial type disloc.

- b) edge dislocation e) precipitate of impurity atoms h) substitutional impure atom
- c) self interstitial atom
- f) vacancy of dislocated loop

Defects make materials more rigid as shearing (bröckeln) is impeded (verhindert). It reduces stability and can also alter properties = doping.



Ritonavir

Ritonavir was originally dispensed as an ordinary capsule, which did not require refrigeration. This was as a crystal of what is now called form 1.18 However, like many drugs, ritonavir exhibits polymorphism, i.e., the same molecule crystallizes into more than one type of crystal. The different crystals, or polymorphs, are made of the same molecules but in different crystalline arrangements. The solubility and hence the bioavailability is very different in the two different arrangements.[19] During development (it was introduced in 1996), only the polymorph now called form I was found, but in 1998, a lower free energy, more stable polymorph (form II) appeared. This more stable (and so less soluble) crystal form compromised the oral bioavailability of the drug. This caused the remoxal of the oral capsule formulation from the market.[19] Even a trace of form II can datalyse the transformation from the more bioavailable form I to form II. Thus form II threatened existing supplies of ritonavir as the lower solubility polymorph caused the therapeutically effective polymorph to convert to form II. Form II, which was not therapeutically effective because of poor solubility and resulting much lower bioavailability, entered production lines and effectively halted production processes.[18] After this discovery in the late 1990s, Abbott (now AbbVie) withdrew the original capsules from the market, and recommended people switch to Norvir suspension while researchers worked to solve the problem. The capsules have been replaced with refrigerated gelcaps, to solve the crystallization problem of the original capsules. In 2000 Abbott (now AbbVie) was awarded approval by the FDA for a tablet (called lopinavir/ritonavir) which contains ritonavir that does not require refrigeration.[20]

Co-crystals are another way to prove properties of a molecule. Different molecules come into the lattice (e.g. NaCl). Materials often used are caffeine, urea (Harnstoff), ...

Co-crystals are distinguishable from traditional pharmaceutical solid-state forms (crystalline, amorphous, solvate, hydrate). Polymorphs only contain the API (active pharmaceutical ingredient) whereas co-crystals contain a **neutral guest compound** = conformer too (nonionic interactions).

 \rightarrow higher bioavailability \rightarrow processability

 \rightarrow higher stability \rightarrow diverse arrays possible

Salt forms

From 21 new chemical entities approved by FDA, 10 were salts. An ionisable drug is combined with a **counter-ion** to form a neutral complex.

- \rightarrow increases chemical stability
- \rightarrow easier administering
- \rightarrow manipulation of pharmacokinetic profile possible



Hardness = ability to move on part of the lattice with respect to another.



→ <u>Temperature effects</u>

By heating the sample, temperature responses have to be figured out: melting point, freezing point, evaporation, sublimation, glass transition temperature, ...

- \rightarrow Thermogravimetric Analysis (TGA)
- → Differential Scanning Calorimetry (DSC)
- \rightarrow Isothermal Microcalorimetry (ITC)

→ Hot Stage Microscopy (HSM)

- Thermogravimetric Analysis (TGA) temperature change = mass change, usually done as a first step: identification of water, chemical decomposition and evaporation.
- 2. Isothermal Microcalorimetry (ITC)

The **heat flow** is generated by an arbitrary sample (willkürlich) chemical, physical or biological process being monitored. (in calorimeter) The sample is kept at a constant temperature. It measures how much heat is needed. The heat flow happens faster with higher temperatures.

- 3. Differential Scanning Calorimetry (DSC)
 - \rightarrow melting (endothermic)
 - \rightarrow glass transitions
 - \rightarrow crystallization (exoth.)
 - \rightarrow decomposition (exoth.)
 - \rightarrow dehydration, desolvation (endothermic).

Glass transition temperature: is a reversible transition in amorphous material from a hard and glassly state into a viscous or rubbery state as the temperature increases. -> result of increasing molecular diffusion, side chain melting

4. Hot Stage Optical Microscope

→ Liquid crystals

They show structural, mechanical and optical properties intermediate to those of crystalline solids and the amorphous, liquid state of matter.

Polymorphic structures are called mesophases.

- \rightarrow Lyotropic LCs: form in presence of a liquid, dependent of temperature and concentration
- \rightarrow <u>Thermotropic</u> LCs: form due to temperature changes



→ <u>Amorphous state</u>

= are non-crystalline materials which posess no long range order

The dosage forms don't have good long term stability since it is the most energetic form. Their structure is like that of a frozen liquid. The degree of crystallinity depends on the fraction of crystalline material in the mixture.

How is amorphous material formed?

- \rightarrow vapor condensation
- \rightarrow supercooling of a melt
- \rightarrow precipitation from a solution
- \rightarrow milling and compaction of crystals

If chemically and physically stable, amorphous materials can have some advantages over the crystalline phase \rightarrow **Novobiocin**: 10 times more soluble and therapeutic active than crystalline form.

→ <u>Hygroscopicity</u>

Compounds and salts are sensitive to the presence of water, vapor or moisture. They retain (behalten) the water by:

- \rightarrow bulk or surface adsorption
- \rightarrow capillary condensation
- \rightarrow chemical reaction

 \rightarrow solution (**deliquescence**) = solid dissolved + saturates a thin film of water on its surface The opposite is **efflorescence**: The crystal loses water below a critical water vapor pressure.

Class 1: Non-hygroscopic	Essentially <mark>no moisture increase</mark> s occur at relative humidities b <mark>elow 90%.</mark>	
Class 2: Slightly hygroscopic	Essentially no moisture increases occur at relative humidities below 80%.	
Class 3: Moderately hygroscopic	Moisture content does n <mark>ot increase mor</mark> e than 5% after storage for 1 week at relative humidities <mark>below 60</mark> %.	
Class 4: Very hygroscopic	Moisture content increase may occur at relative humidities as low as 40 to 50%.	

!!!Skript!!!

→ Gas or vapor adsorption

Dynamic Vapor Sorption (DVS)
 Increasing humidity = change in mass (provides insight in mechanism of bound water)

Adsorption of gas molecules:

 \rightarrow Adsorption is the accumulation of atoms or molecules on the surface of a material. It creates a film of the adsorbate.

 \rightarrow Absorption: A substance diffuses into a

- liquid of solid to form a solution.
- \rightarrow Sorption encompasses both processes.
- \rightarrow Desorption is the reverse process.



Brunauer's model of multilayer adsorption

/ Inselwachstum (Volimer-Weber	Mischform: Insel- und Schicht-für-Schicht = Stranski-Krastanov	Schicht-für-Schicht (Franck-van der Merwe)
0<1ML	minin	TITITIT
Bedeckungsgrad kleiner als 1 Monolage (monolayer)		
1<0<2		711111111
θ>2	clusters or crystals	7//////////////////////////////////////

2. Surface Analysis (BET) - 1938

BET (Brunauer, Emmett, Teller) theory aims to explain the physical adsorption of gas molecules on a solid surface. The analysis method can be used to determine: Adsorption, surface area (can correlate with dissolution, measure of **how much exposed area** a solid object has), density and porosity.

Isotherms

= amount of adsorbate on adsorbent as a function of its pressure (gas) or concentration (liquid) at **constant temperature** \rightarrow comparison of different materials

by Freundlich/Küster, Langmuir and Brunauer/Emmett/Teller

The surface of the adsorbent is uniform = all adsorption sites are equivalent. Adsorbed molecules **do not interact lateral**. At the maximum adsorption, only a **monolayer** is formed.





Adsorption is also visible in **solution** (polymers):



<mark>→X-Rays</mark>

Absorbance of x-rays depend on material property.

$$I = I_0 * exp(-\beta L)$$

 βabsorption coefficient L....length in sample

Computer tomography (CT):

- \rightarrow X-ray images from various sides
- \rightarrow non invasive
- \rightarrow radiation level high: illumination of spinal cord
- \rightarrow brain is much less affected by radiation
- \rightarrow no impact of specimen (Proben)
- \rightarrow no need to cut sample open

X-Ray diffraction = reflection on net-planes

Net-planes are artificial planes which contain a periodic

structure (crystal). If they lead in one direction, they have the same separation (= **d-spacing**). **Bragg's law:**

$$\lambda = 2d_{hkl}\sin(\frac{2\theta}{2})$$



λ...wavelength of x-rayd...d-spacingsθ...angle of incidence

Incident angle = exit angle

X-rays interact with **every electron** of the atom = **Thomson scattering**. Every electron emits a spherical wave which interferes at some points in space. Constructive interference is explained by the **Laue-equations**.



Diffraction is measured with a "Kristalloflex powder diffractometer".

Electric charge changes velocity and direction of the beam. Electrons are shot out of the inner core shell \rightarrow X-ray **fluorescence**. For structure evaluation X-rays of 1 – 2.5Å are used. In hospitals, they have 8-10 times more energy or a shorter wavelength.

X-Ray diffraction light source

Synchroton = large light source which provides much faster measurements, higher resolution, but the sample may be degradet

X-Ray powder diffraction (XRPD)

- small crystallites (100nm $5\mu m$) with random orientation
- all net-planes are accessible

→In situ XRPD

- polymorph identification (are only stable at elevated Temperatures)
- slow compared to DSC or Light microscope
- phase transition may be slow

→Qualitative phase analysis X-ray powder diffraction

- -measure XRD pattern over maximal range
- identifies peaks
- comparison with known materials/polymorphs via databases (Campridge structure db)
- when it's not in a database -> Rietveld refinement or single crystal measurements

\rightarrow Quantitative phase analysis with X-ray powder diffraction

- measure whole accessible range
- evaluates peak areas for each polymorph
- important to take amorphous fractions into account

→Small angle X-Ray diffraction

- we get the particle size, shape, separation and interaction as well as the inner surface
- identification of large d-spacings (lipids and micelles)

→Problems with XRPD

- Variation in particle size can lead to non-random orientation if the particles are too large and if the particles are too small it will lead to broadening of the diffraction peaks. (may appear amorphous) $1\mu m$ >...<10 μm

-Prefferd orientation; when the powder consists of needle or plate-shaped particles these tend to become aligned parallel to the specimen axis, therefore the sample is usually rotated.

-Statistical errors; to prevent these scanning should be carried out at an appropriated slow speed

SAXS provides information abourt the overall size and shape of the particle (small angles) WAXS provides information on the phase state and the crystal symmetrie (large angles) TRUE WAXS simultaneously wide and small angle measurements – complete information

→Neutron scattering and diffraction

- Scattering/diffraction of neutrons on periodic structures
- Interaction of neutron with nucleus
- Intereaction is weak -> low contrast/flux requires long measurement time
- Advantage of neutrons is they highlight regions in sample by changing hydrogen atoms to deuterons (contrast variation)

→Free electron laser

Exceptionally bright and fast X-rays can image proteins using x-ray crystallography. This technique allows first-time imaging of proteins that do not stack in a way that allows imaging by conventional techniques, 25% of the total number of proteins. Resolutions of 0.8 nm have been achieved with pulse durations of 30 femtoseconds. To get a clear view, a resolution of 0.1–0.3 nm is required. The short pulse durations allow images of x-ray diffraction patterns to be recorded before the molecules are destroyed. The bright, fast Xrays were produced at the Linac Coherent Light Source at SLAC. As of 2014 LCLS was the world's most powerful X-ray FEL.[27]

Particle size distribution curve: the number, or weight of particles within a certain size is plotted against the size range or mean particle size – more interested in weight distribution Determination with works by Edmundson, Allen and Groves or with Sieving

→Light scattering e.g. Static light scattering

intensity of diffracted light depends on the angle of incident and scattered angle -> interference

- measure signal fluctuation at one angle, calc autocorrelation function

we can learn the particle size, size distribution, polymer coil size, Proteine size, diffusion coefficient and solvent quality

-measure signal fluctuation at one angle, vary electric potential

we can learn surface charge of particles and variation of pH due to iso-electrical point

→Atomic force microscopy

Shows surface of DNA, Chromosome and Cells - Confinement at the solid-liquid interface through

- identifyieng layering
 - the electrostatic repulsion is stable
 - van der Waals attraction is unstable

Single polymer chain stretching - polymer can attaché to silica surface and colloid sample - polymer bridge can be strained - single molecule stretching allows identification of polymer solvent interaction





Flex-FPM opens the door to fascinating new experiments



→Scanning electron microscopy SEM

enables the investigation of specimens with resolution down to nanometer scale. Operates in high vacuum. An electron beam is generated by an electron cathode and swept across the surface of the sample. The magnification is computed by the ratio of the image in form of a raster. The signals which are generated are primary electrons (PE), secondary electrons (SE) and backscattered electrons (BSE) and furthermore x-rays



An indirect usage is to use electrons to generate light and a disadvantage is the requirement of covering the surface with conducting material

→Environmental scanning electron microscopy

Has two futher vacuum states which allows to get information about the tensile stage, heating stage and Peltier cooling stage, by using a low vacuum mode and the ESEM mode (investigation of wet samples by cooling them down)

→Transmission electron microscopy

More recently, advances in aberration corrector design have been able to reduce sphericalaberrations[58] and to achieve resolution below 0.5 Ångströms (50 pm)[56] at magnificationsabove 50 million times.[59] Improved resolution allows for the imaging of lighter atomsthat scatter electrons less efficiently, such as lithium atoms in lithium battery materials.[60]The ability to determine the position of atoms within materials has made the HRTEM_an indispensable tool for nanotechnology research and development

in many fields, including heterogeneous catalysis and the development of semiconductor devices for electronics and photonics. !!!Fußzeile!!!



Dosage Form Considerations

Preformulation studies inevitably extend beyond the basic characterization of the lead compound, because what is considered as an acceptable characteristic of a lead compound will largely depend on the intended or anticipated dosage form. For example, the solubility issues will largely determine the route of administration; conversely, if a particular route of administration is the only desired route, then preformulation studies should attempt to find out the structural changes necessary for the candidate molecule.

Dosage Form depends on:

- o Rate of entry to body tissues desired
- Onset of action desired
- Solubility (aqueous non aqueous)
- Irritability of solution of drug
- Stability of drug
- Storage and handling for dosage form
- Shelf life
- Patient acceptance



Bioavailability -

Stability

Technical Feasibility

Equilibrum between the bioavailability of product, its chemical and physical stability and the technical feasibility of producing it.

→Solid dosage Form considerations

- Most new drugs enter market in form of tablets or capsule, because it is the least invasive method, it is understand and accepted by patients and easily to administer for them
- They are also cheap to manufacture and provide a staple and compact form
- o The solid dosage form is manufactured from powders
- Important powder properties:

-Particle size and shape -Density/Compressibility -Flow: different movement due to cohesive forces between particles (van der Waal), electrostatic charging and moisture forces (capillary force) -measurement of flow via shear cell method, angle of responds, avalanching behaviour -Mixing behaviour -Compaction

- Taste and appearance are important
- Dissolution is very important
- Properties for tablet formulation are compatibility of drug substance with excipients, flowability, compactability, lubricity, appearance, disintegration, dissolution



→ Parenteral dosage form considerations

- Means all routes except oral and is often done via injection
- Routes are intravenous, subcutan (1-1.5ml) and intramuscular (2ml) small volume paenterals <100ml, large volume parenterals >100ml
- pH value (7.4) can change the local pH value. Better not to use buffer, due to interaction with physiological buffer system isotonic is essential for fast transport for LVPs Tonicity (osmotic pressure gradient)
- daily dose of excipient must be taken into account for formulation (co-solvents, surfactants, complex agents)
- sterility is the utmost important

→Inhalation dosage form considerations

- Asthma, bronchitis and emphysema are therapied with steroids and short and long acting b2s
- Inhalation allows the delivery of doses direct to the lungs via dry powder inhaler (DPIs), metered dose inhalers (MDIs) and nebulizer.
- Pulmonary delivery is appropriate with cystic fibrosis, HIV, lung cancer and also for the treatment of non-respiratory diseases e.g. insulin
- Particles are deposited in the lung through Impaction, Sedimentation and Diffusion. It varies on the airflow and disease state.

→Topical dosage form considerations

- Used dermatological, local and transdermal
- The skin structure is a complex cell structure and protective against the environement
- Permeability of the skin is defined by concentration of substance applied, partition coefficient and diffusion within skin.
 - also different with skin regions and ethnical background
- Penetration enhancer are water, organic solvents, ester...
- Formulations types are gels, emulsions... (drug can be in matrix or in reservoir, or in adhensive)
- Injection with microneedles
- Iontophoresis, electroporation, non-cavitational ultrasound, jet injections, dermabrasion, thermal ablation

Indivual formulations due to gender, age and heritage would be better for therapeutic actions. Therefore the goal of preformulation is to meet the individual needs.