DISSOLUTION & ABSORPTION

2020

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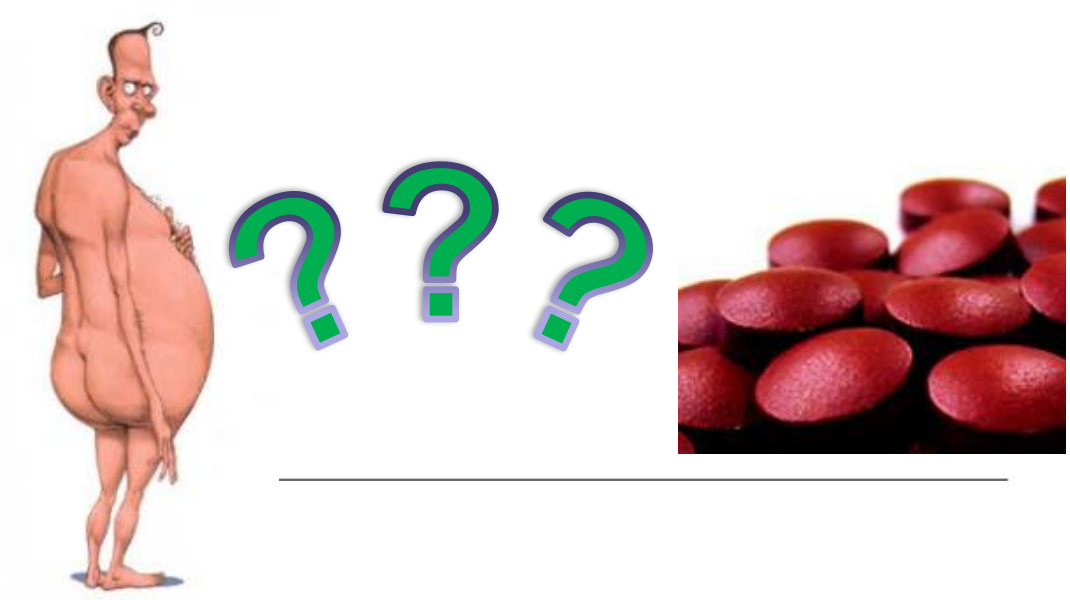
Interaction active ingredient × organism

- **pharmaceutical phase – disintegration, dissolution (pharmaceutical availability)**
- **pharmacokinetic phase – ADME (biological availability), AUC, c_{max}, t_{max}**
- **pharmacodynamic phase – interaction between drug and receptor (therapeutic effect)**



Bioavailability

oral administration

disintegration

dissolution

ingredient can diffuse

absorption

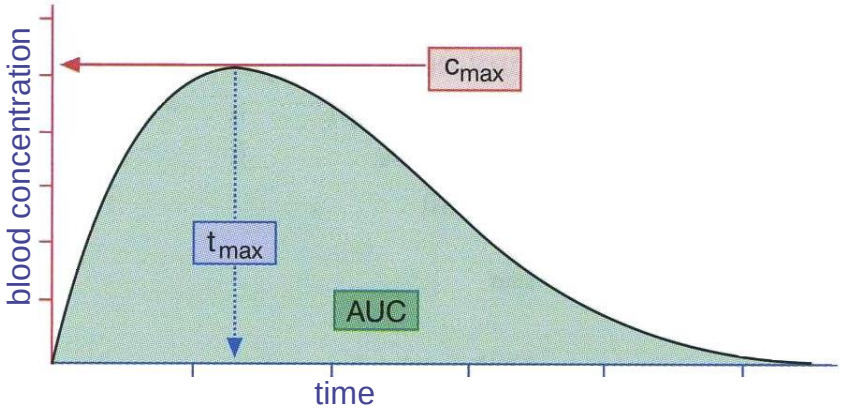
hepatic pass

systemic distribution

galenic availability

resorption part

first pass effect



Bioavailability

characterize a ratio between solubility in water and lipids

permeability across membranes

Biopharmaceutical Classification System (BCS)

- **highly soluble API (BCS) – its highest dose is soluble in 250 ml of a dissolution medium in physiological pH range**
- **highly permeable API (BCS)**
  - **– absorption is > 90%**

Permeability high	Class 1 (amphiphilic) tramadol.HCl losartan pravastatin	Class 2 (lipophilic) atorvastatin itraconazole valsartan
Permeability low	Class 3 (hydrophilic) gabapentin metformin.HCl valcyclovir	Class 4 (trouble makers!) acyclovir furosemide cyclosporine
	Solubility high	Solubility low

Biopharmaceutical Classification System (BCS)

BCS class	Solubility	Permeability	Speed limiting item	Dissolution requirements	Note
1	high	high	stomach emptying	fast in all pH range (85% in 30 min in all media)	
2	low	high	dissolution	specification based on IVIVC	absorption controlled by solubility of API
3	high	low	absorption through instestine membrane	very fast in all pH range (85% in 30 min in all media)	fast solubility required to maximize the absorption
4	low	low	dissolution and absorption	low chance for IVIVC	prodrug preparation, higher solubility = higher permeability

Disintegration × dissolution

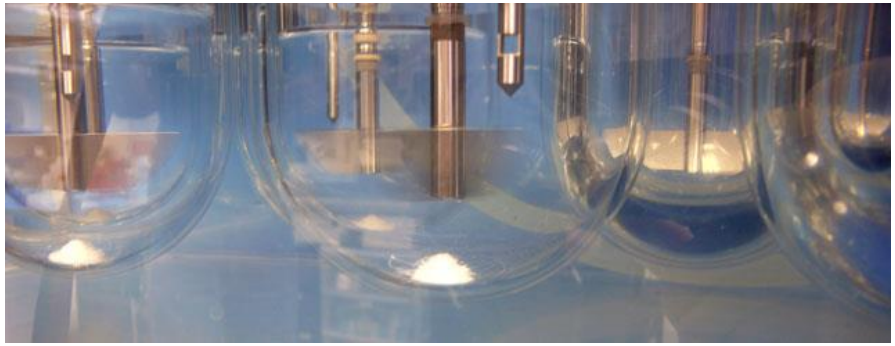
- **disintegration – ability of a dosage form to disintegrate into particles (compression pressure, porosity, excipients)**
- **dissolution – releasing of molecules (ions) from a crystal bond and their diffusion in a solvent or digestive juices (chemical form: salt, weak acid, weak base; physical form: amorphous, polymorph, particle size)**

Disintegration

- **disintegration – studies if tablets or capsules disintegrate under defined experimental conditions in a defined liquid, within defined time**

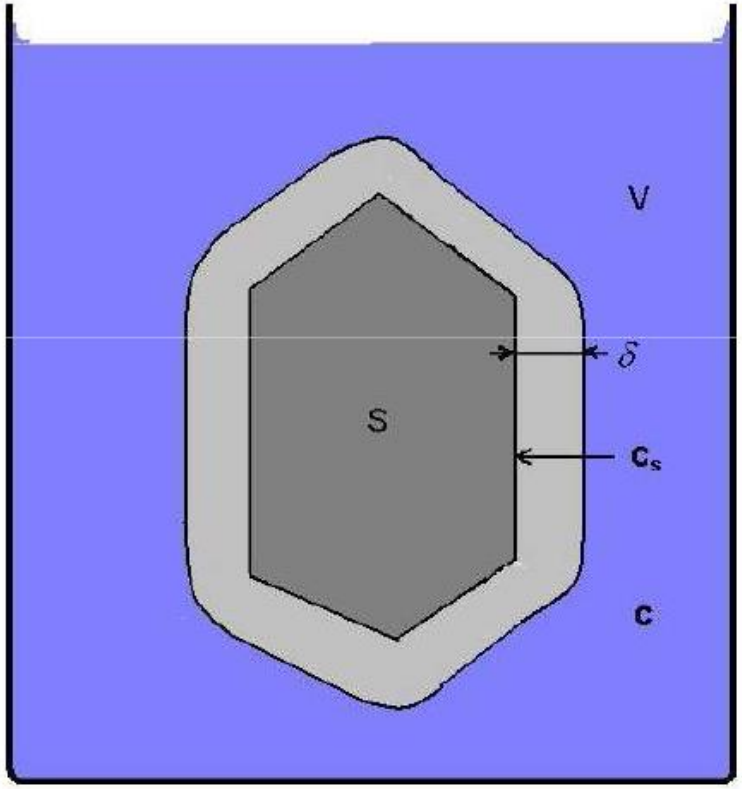
Dissolution

- process, by which a solid substance comes into a solvent and becomes a solution
- necessary requirement for drug absorption
- important tool for proposal, manufacture, evaluation and quality control of dosage forms
- connection of *in vitro* testing and *in vivo* availability



Dissolution of API – Intrinsic dissolution

- **Noyes-Whitney equation**



$$\frac{dn}{dt} = \frac{DS}{\delta}(c_S - c)$$

dn/dt – a mass of a tested substance dissolved per time unit

D – diffusion coefficient

S – area of phase boundary between a solid phase and a solution

c_S – concentration of a saturated solution on a phase boundary

c – concentration in all volume

δ – width of diffusion layer

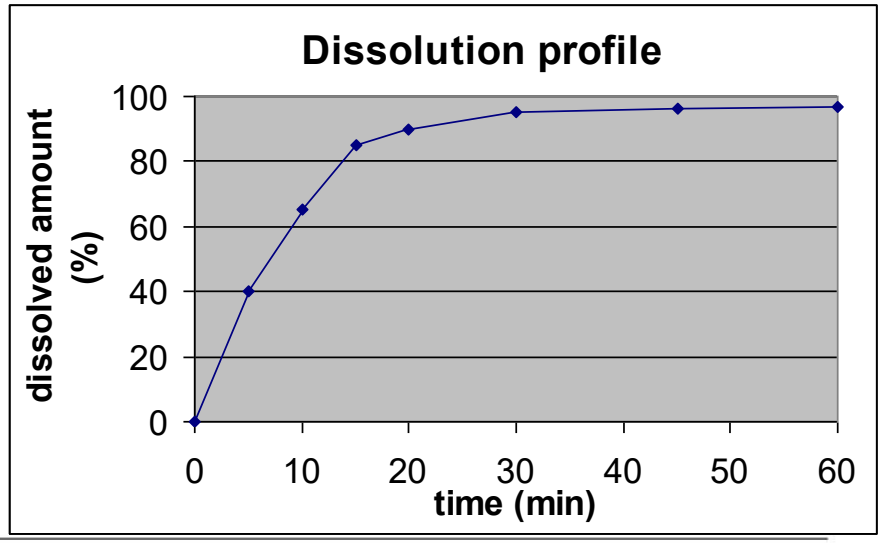
Dissolution of API – Intrinsic dissolution

- pure API or a mixture with excipients
- critical parameter – preparation (compression of a tablet)

Dissolution modifying factors		Physicochemical properties of API	Physiological factors in GIT
diffusion coefficient	D	molecule size M_r, temperature	viscosity of digestive juices
surface area	S	particle size, wettability	surfactants, bile
width of diffusion layer	δ	——	motility, flow rate
solubility	c_S	hydrophilicity, crystal structure, solubilisation	pH in GIT, bile, food, buffer capacity

Dissolution – Dissolution profile, specification

- **dissolution profile is a dependence of released amount of active substance on time**
- **specification of dissolution is specified by minimal acceptable amount of released active substance within specified time (minimal XX % within YY min)**



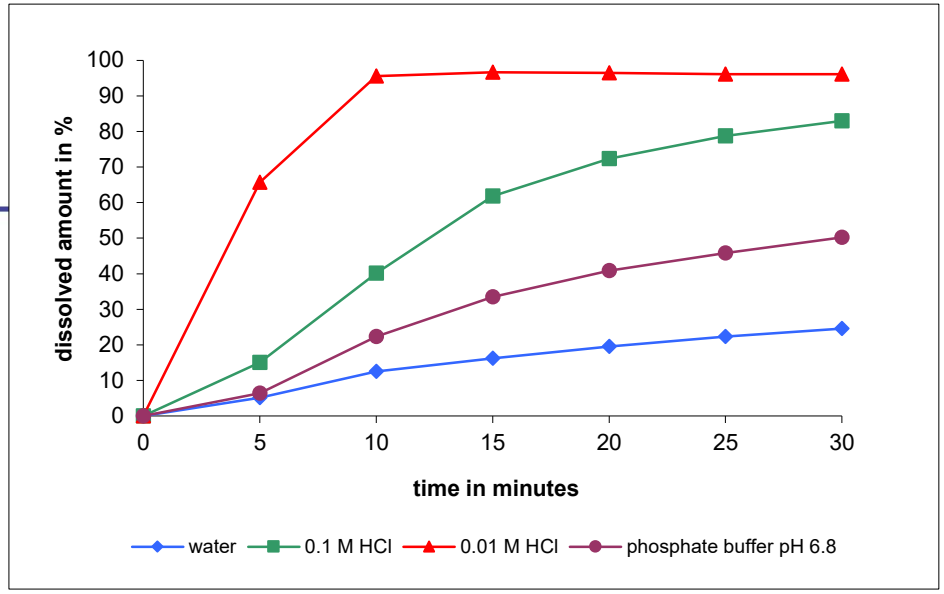
Rate of active substance releasing from a dosage form

- **physicochemical properties of API (solubility, polymorphy, size and shape of particles, porosity)**
- **formulation components (excipients, buffers, surfactants)**
- **manufacturing process (mixing process, wet granulation, melt extrusion, tableting pressure)**
- **physical properties of a dosage form (wettability, swelling, disintegration)**

Rate of active substance releasing from a dosage form

- **dissolution test**
  - **media composition, pH, ion power, buffer capacity**
  - **media volume**
  - **stirring (hydrodynamics)**

Dissolution media



- temperature: (37 ± 0.5) °C
- buffer pH: 1 to 6.8 (± 0.05)
- volume: 500 – 1000 ml
  - saturated concentration determination, sink conditions calculation $c_S/c_d > 3$
- additives: surfactants, enzymes
- rotation speed: 50 – 100 rpm

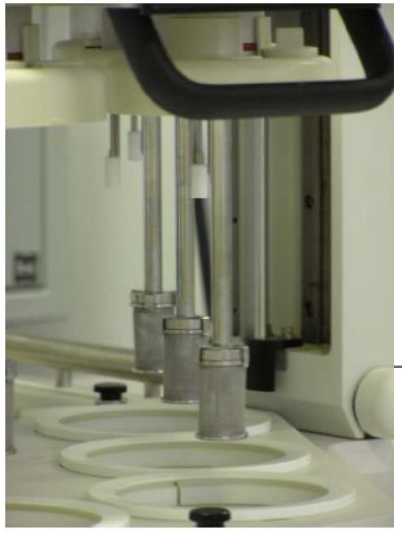
Dissolution methods

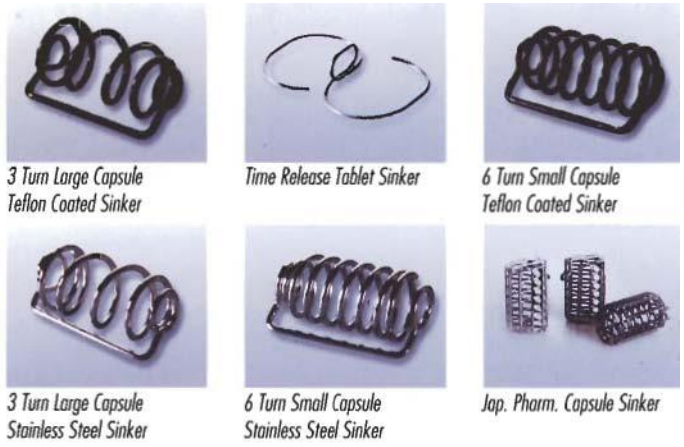
- 7 types according to the USP
  - baskets
  - paddles
  - reciprocating cylinder
  - flow-through cell
  - paddle over disk
  - rotating cylinder
  - reciprocating holder
  - special instruments (TNO TIM-1, „Golem“)
  - on-line
  - off-line

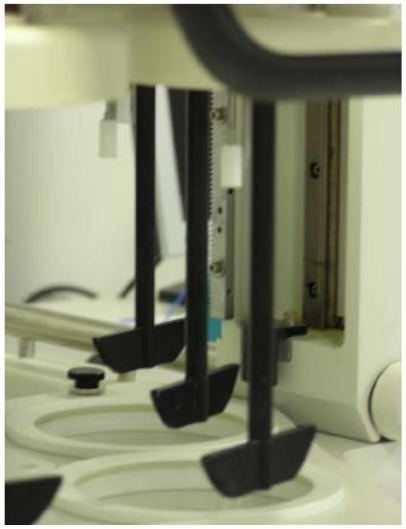
Dissolution methods – USP 1, 2

- 1 – basket, 2 – paddle

- limitation – traditional closed models, dissolved drug stays in the system – accumulation × absorption *in vivo*, concentration gradient is establishing
- flowing on a surface, sticking on a bottom (spirals), different hydrodynamics in particular parts of solution

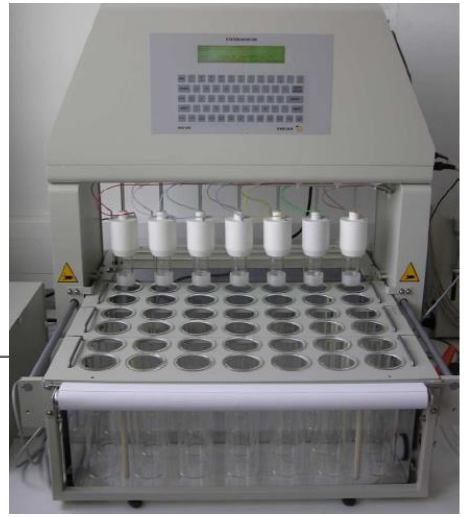






Dissolution methods – USP 3

- **USP 3 – equipment with reciprocating cylinder, closed system**
- **simpler simulation of GIT conditions (pH changes and transit times)**
- **product releasing in 6 different pH media**
- **suitable mainly for MR products, proof of drug form against strong mechanic stress**

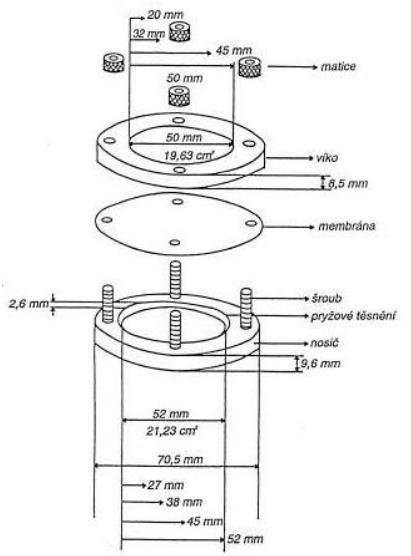


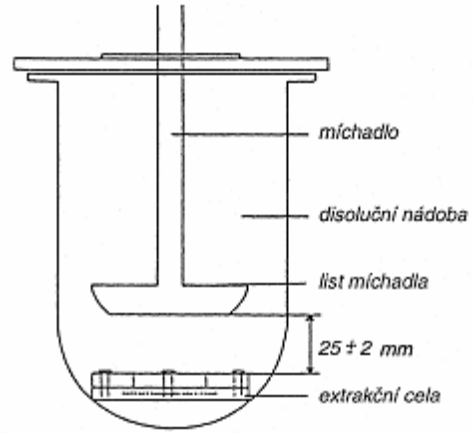
Dissolution methods – USP 4

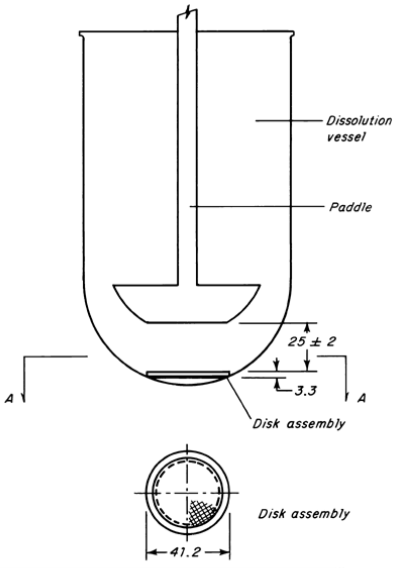
- **USP 4 – flow-through cell, open system**
- **absorption process is simulated by keeping of concentration gradient, no peristalsis**
- **suitable mainly for products containing low soluble API, MR products, special dosage forms**

Dissolution methods – USP 5

- **paddle over disk, extraction cell**
- **for transdermal preparations**
- **32 °C**







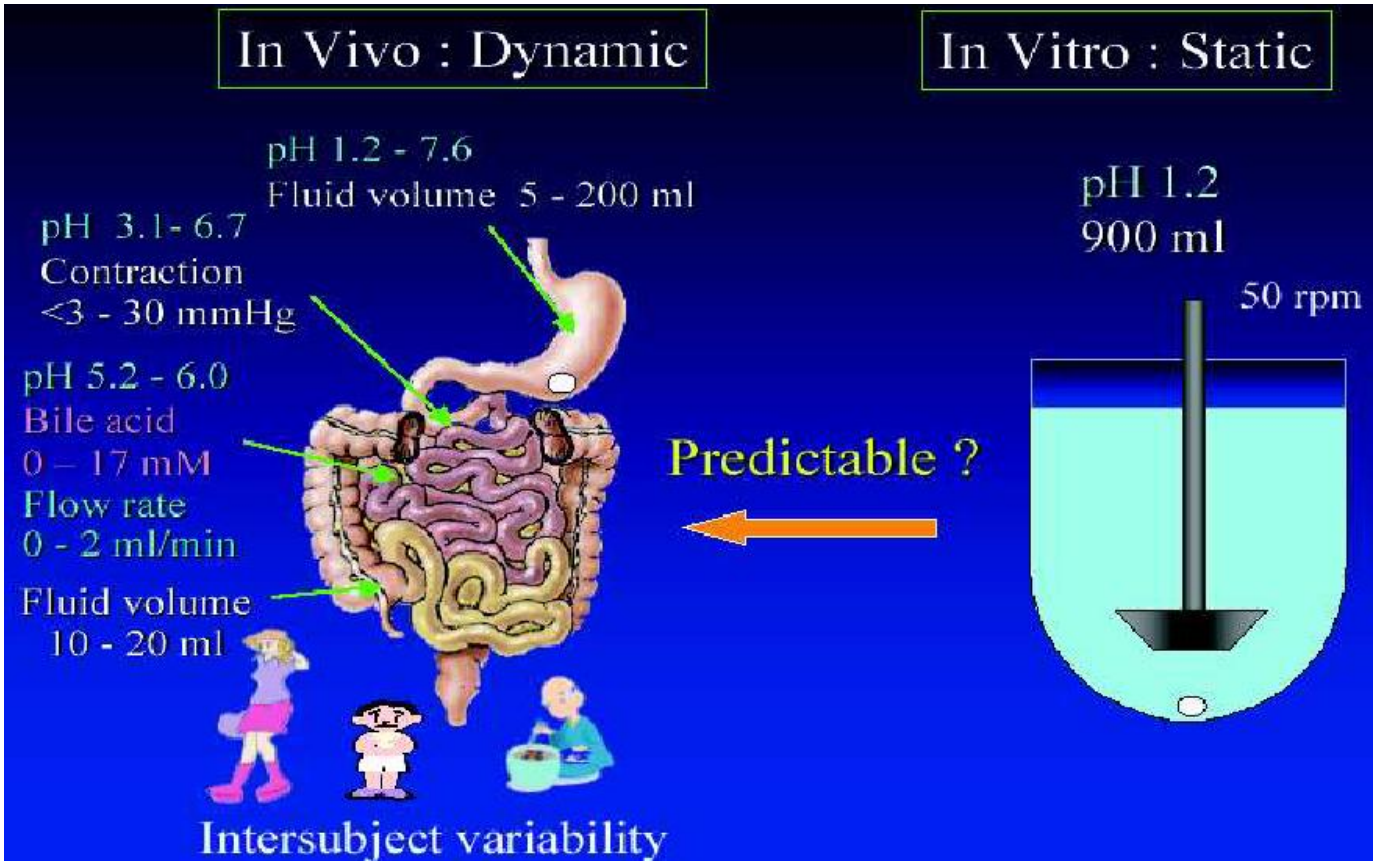
Dissolution methods – USP 6

- **rotating cylinder**
- **for transdermal preparations**
- **32 °C**

Dissolution – Requirements for dosage forms

- **fast releasing dosage forms – minimum 85% within 15 min**
- **immediate releasing dosage forms – minimum 70 – 80% within 30 – 45 min**
- **modified release dosage forms – dissolution lasts usually 8 – 24 h, specification minimum in three points in profile**
- **delayed release dosage forms – enterosolvent tablets**

Dissolution methods – Physiological approaches



Dissolution methods – Physiological approaches

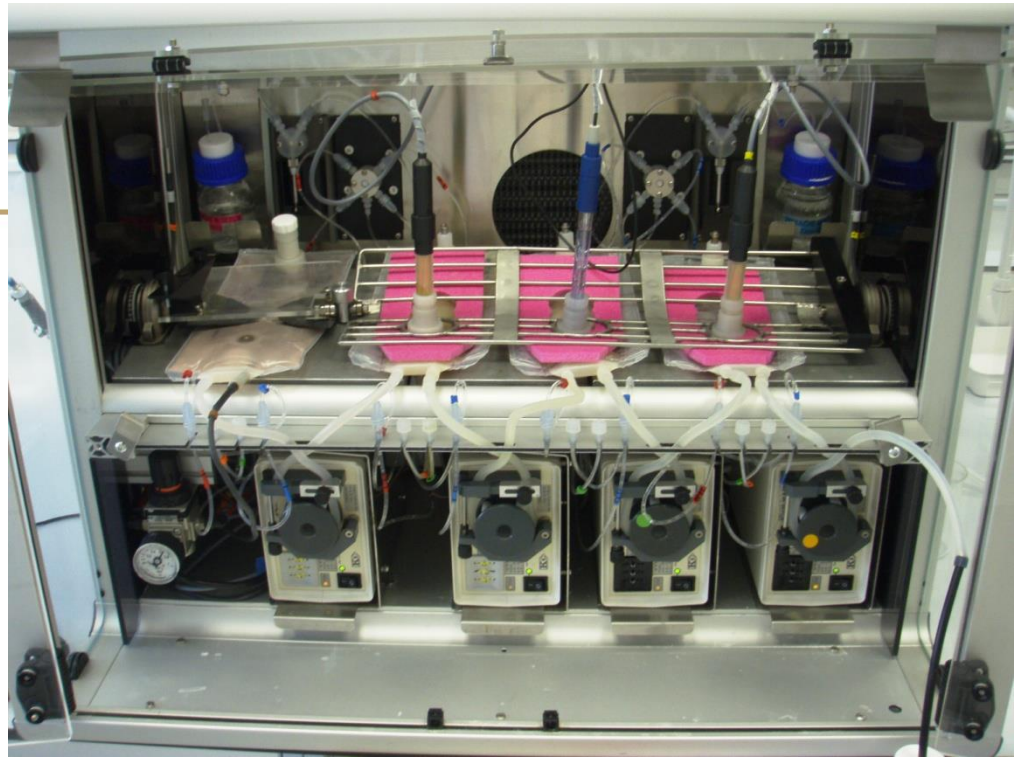
- **pepsin**
- **bile salts**
- **phospholipids (lecithin)**
- **lipase, pancreatic enzymes**
- **Ca^{2+} (lipase activity)**
- **ion power**

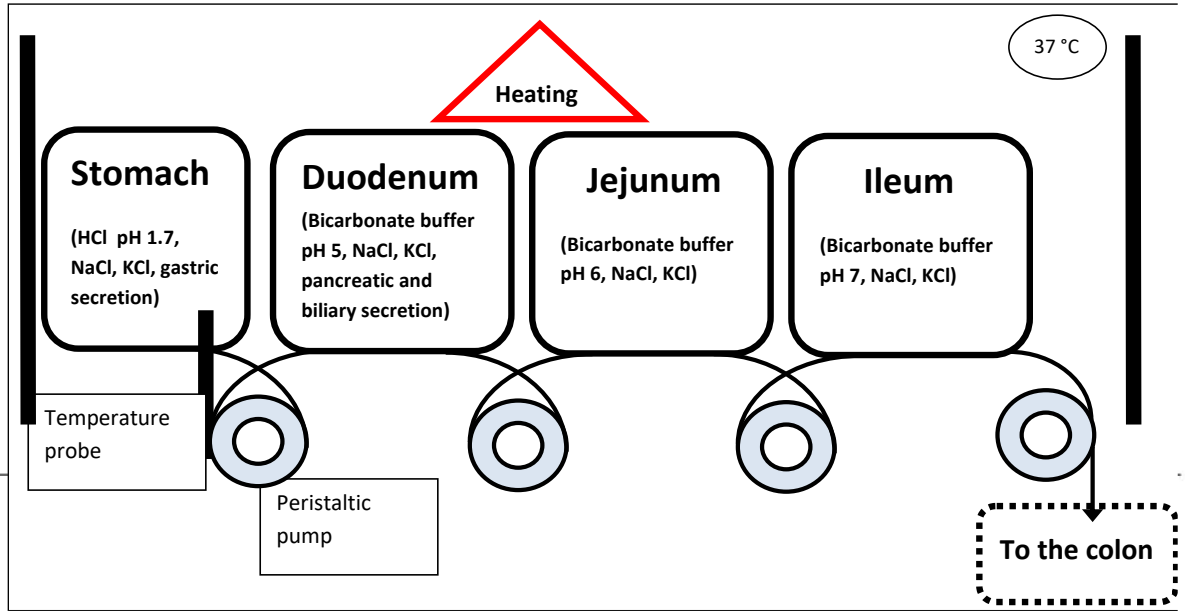
- **Fasted State Simulated Gastric Fluid (FaSSGF)**
- **Fed State Simulated Gastric Fluid (FeSSGF)**
- **Fasted State Simulated Intestinal Fluid improved version (FaSSIF V2)**
- **Fed State Simulated Intestinal Fluid improved version (FeSSIF V2)**

Dissolution methods – Physiological approaches

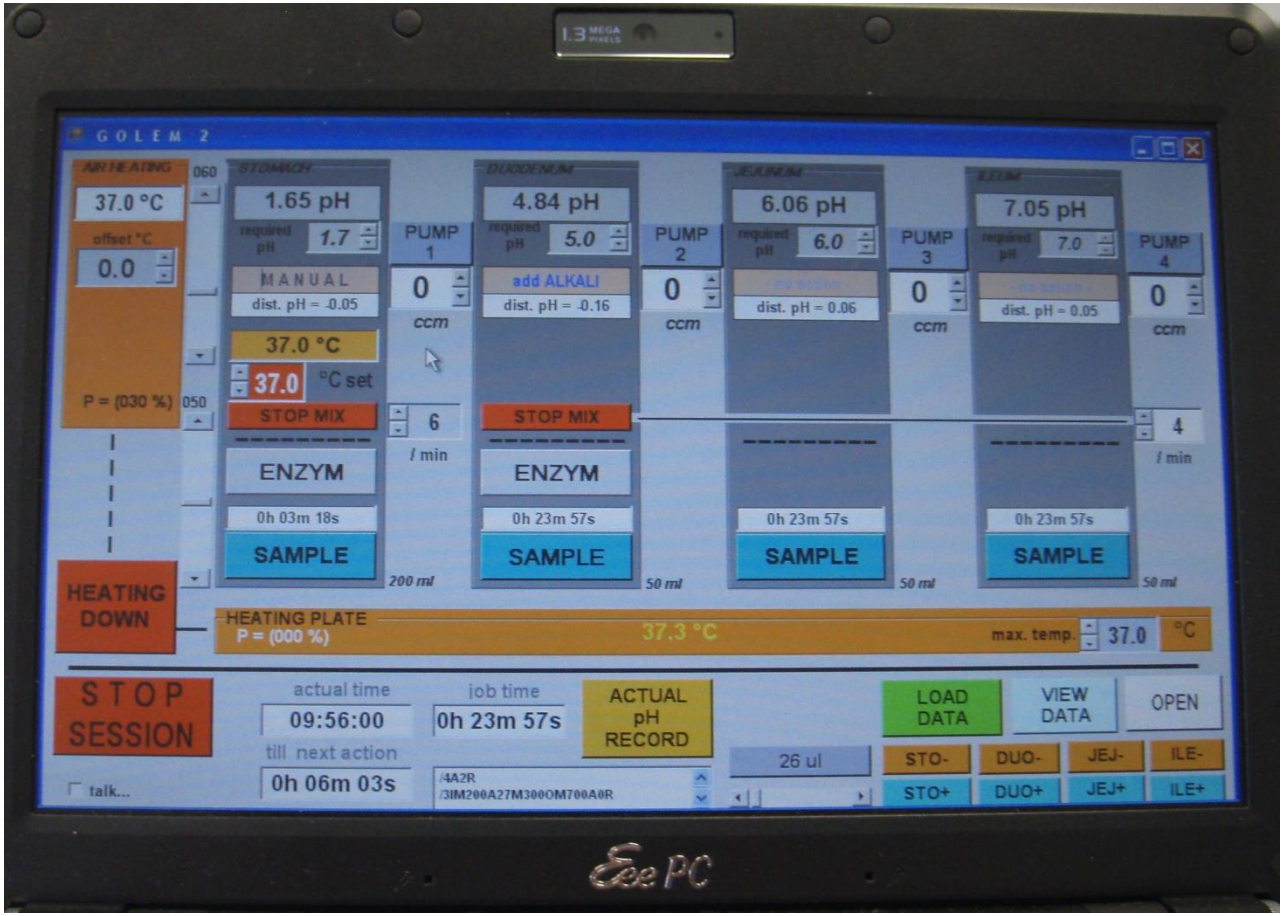
- **gastro-intestinal dissolution model TNO TIM-1**
- **full simulating of GIT (stomach, duodenum, jejunum, ileum, pancreatic and bile secretion, enzymes, dynamic conditions, peristalsis, body temperature, ion power)**

➢ Golem



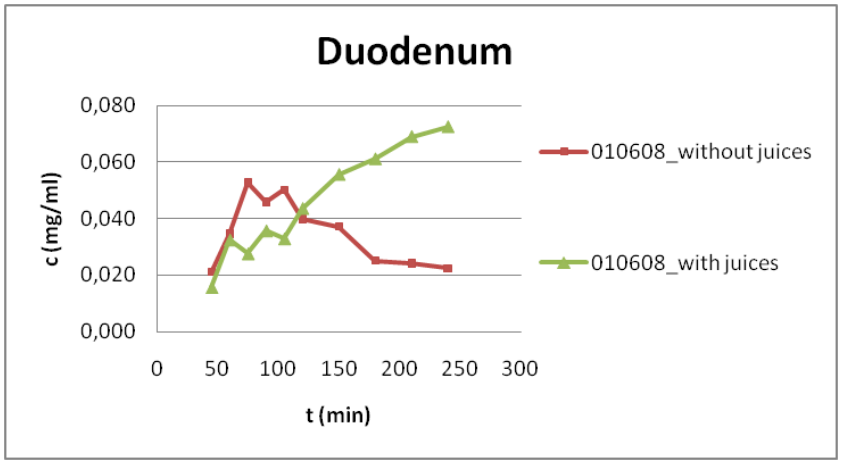

37 °C
Heating
Stomach
(HCl pH 1.7, NaCl, KCl, gastric secretion)
Duodenum
(Bicarbonate buffer pH 5, NaCl, KCl, pancreatic and biliary secretion)
Jejunum
(Bicarbonate buffer pH 6, NaCl, KCl)
Ileum
(Bicarbonate buffer pH 7, NaCl, KCl)
Temperature probe
Peristaltic pump
To the colon

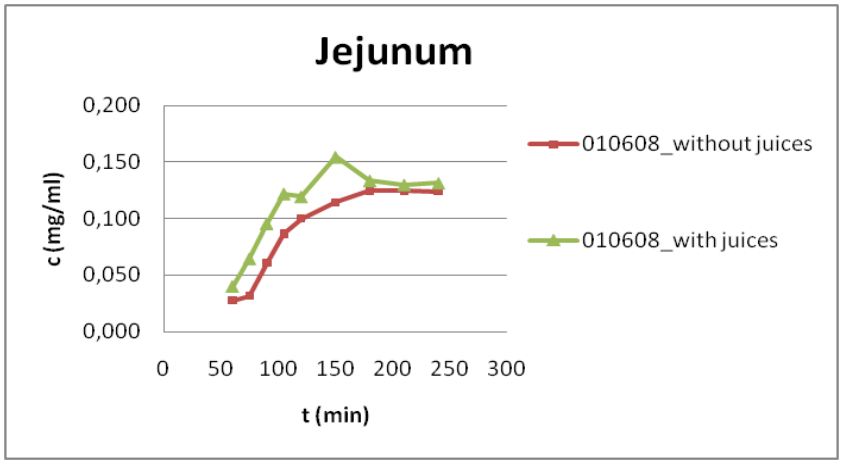
➢ Golem – user-friendly software

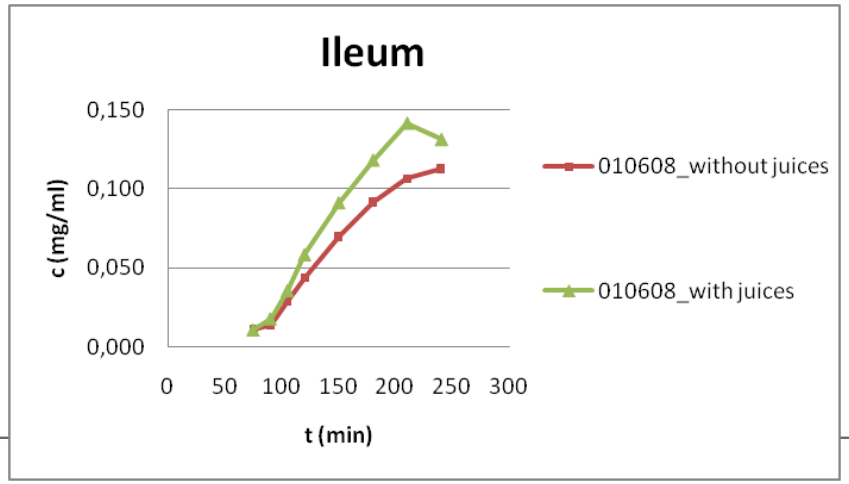


Examples

- **Influence of bile and pancreatic juices (atorvastatin)**

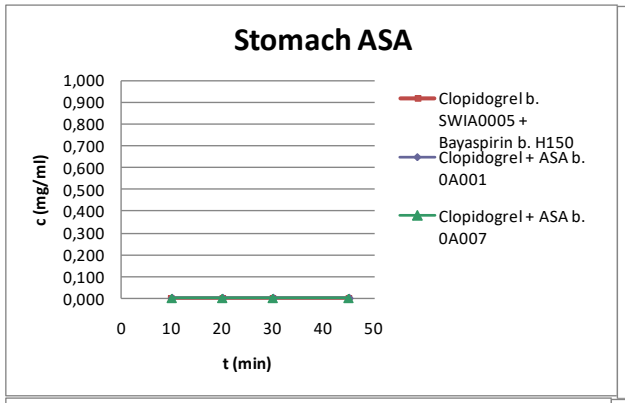


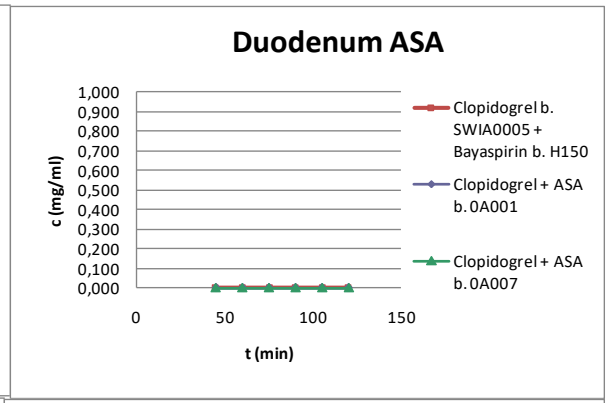


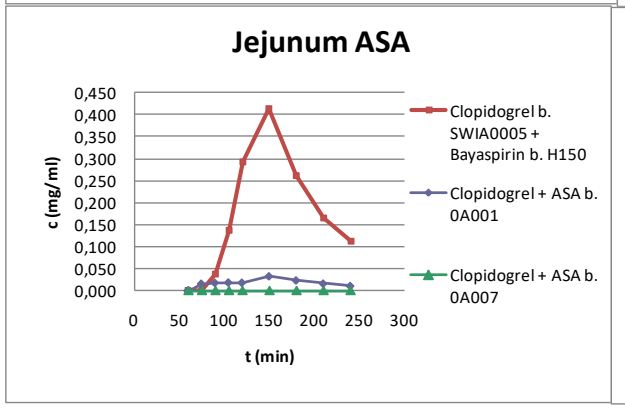


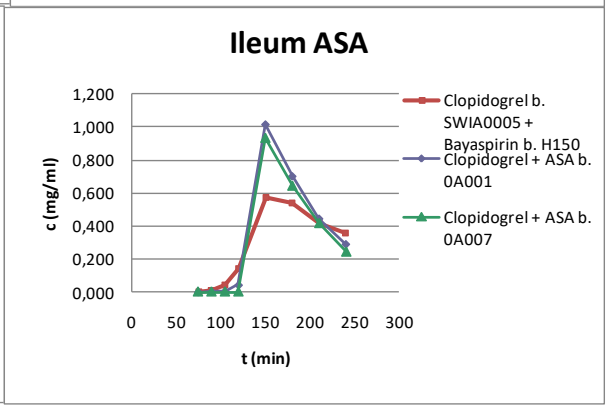
Examples

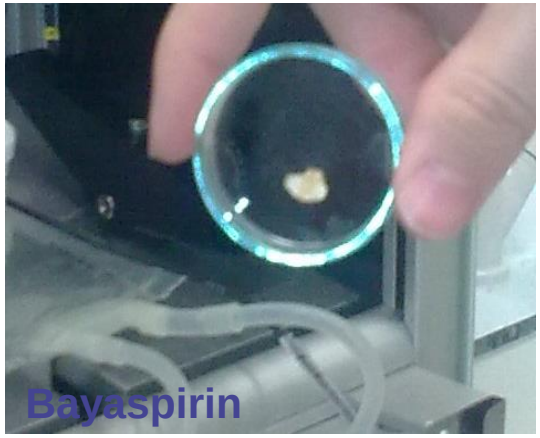
➢ Clopidogrel + acetylsalicylic acid (enterosolvent tablets)





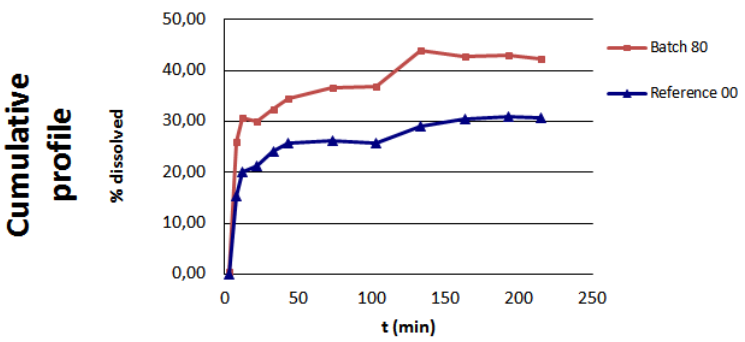


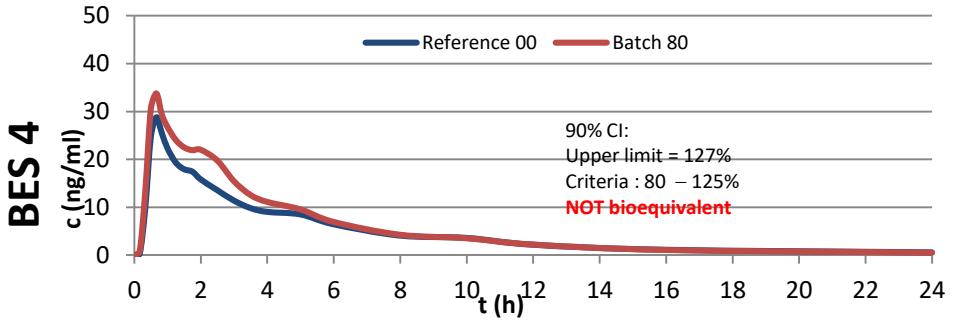


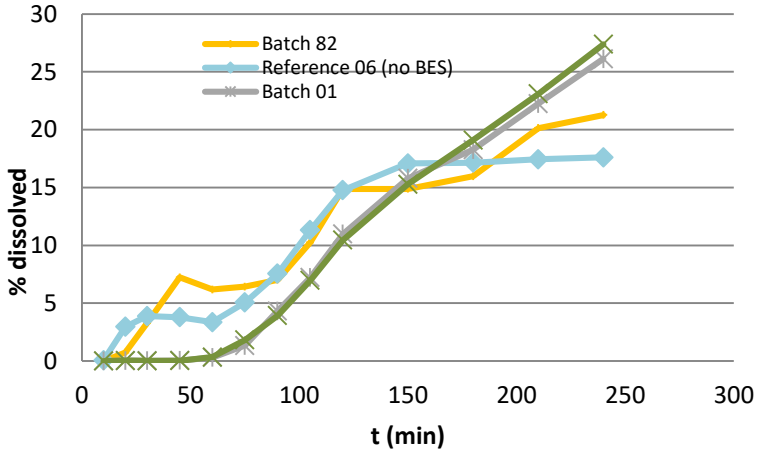


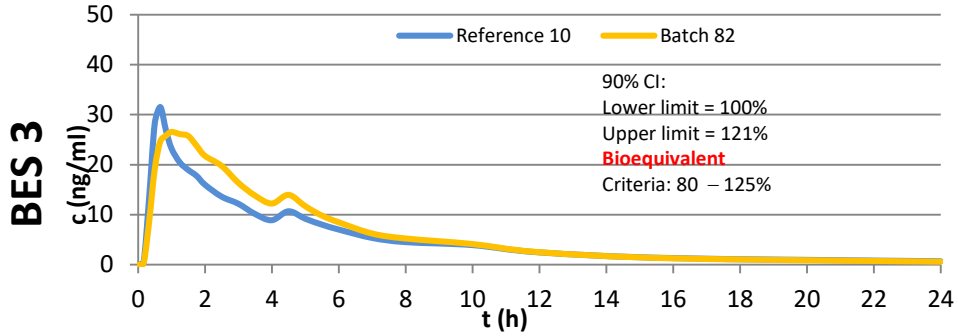
Examples

- **Atorvastatin (batches 80 and 82 vs. reference)**
  /thanks also to Dr. Čulen/





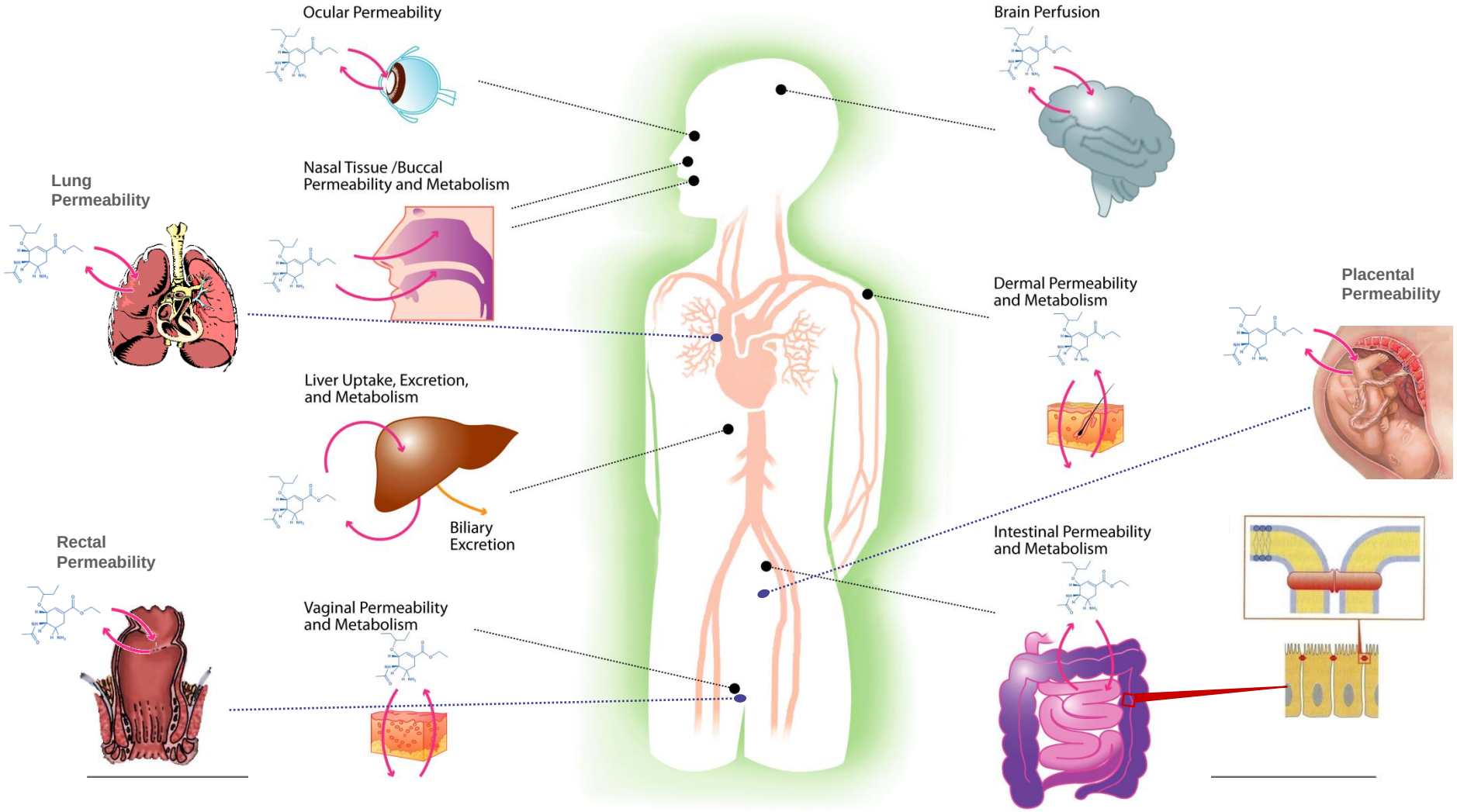




Dissolution methods – Directives, guidelines

- **European Pharmacopoeia Ph. Eur.**
- **Czech Pharmacopoeia – Dissolution of solid dosage forms, Dissolution of transdermal preparations, requirements on particular dosage forms**
- **EMA guideline (European Medicines Agency) – ICHQ2(R1) – method validation (accuracy, precision, linearity, robustness, …)**

Absorption – Methods of administration, barriers



Absorption influencing factors

chemical factors
(lipophilicity, molecule size,
ability to be ionized
mechanism of absorption)

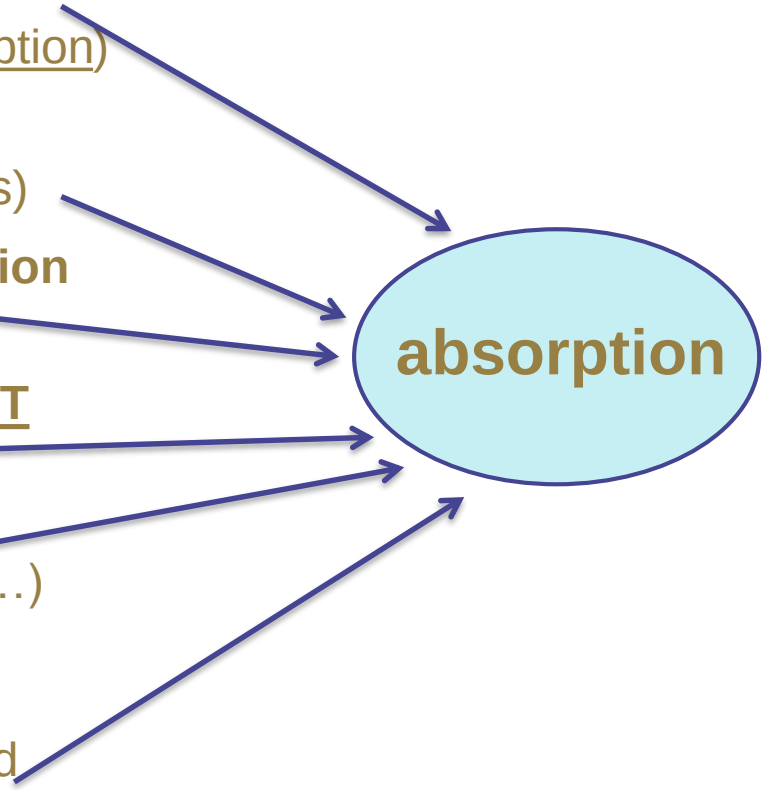
physical factors
(particle size, enhancers)

method of administration
(i.m., p.o., …)

functional status of GIT
(motility, …)

drug interactions
(antacids, adsorbents, …)

formulation factors
(type of dosage form,
excipients, inhibitors and
enhancers of carriers, …)



Absorption influencing factors – Chemical factors

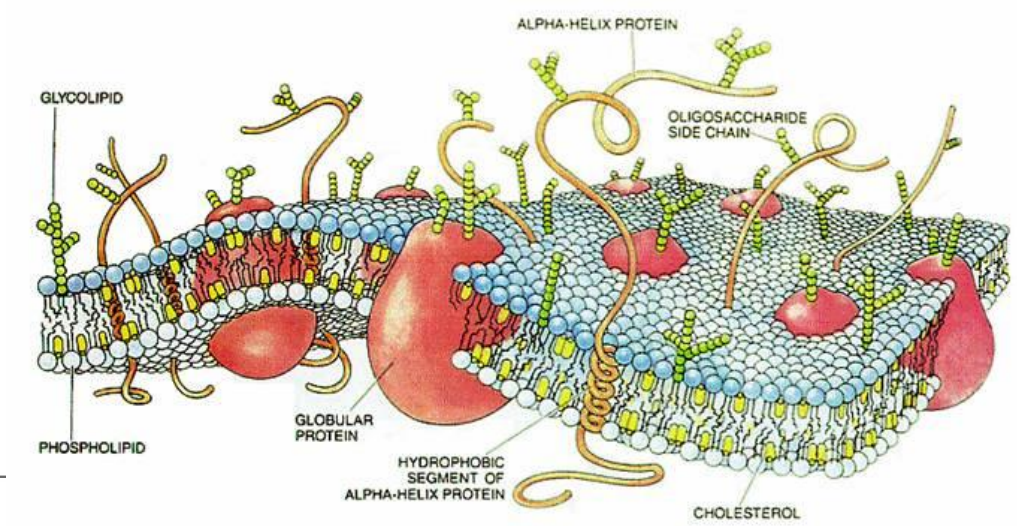
- **ability to be ionized (pK_a)**
  - **important influence on drug solubility and permeability (passive diffusion absorption – only nonionized form is absorbed)**
  - **many drugs are weak acids or bases**
  - **Henderson-Hasselbalch equation**

    for acids: $\log([HA]/[A^-]) = pK_a - pH$

    for bases: $\log([BH^+]/[B]) = pK_a - pH$

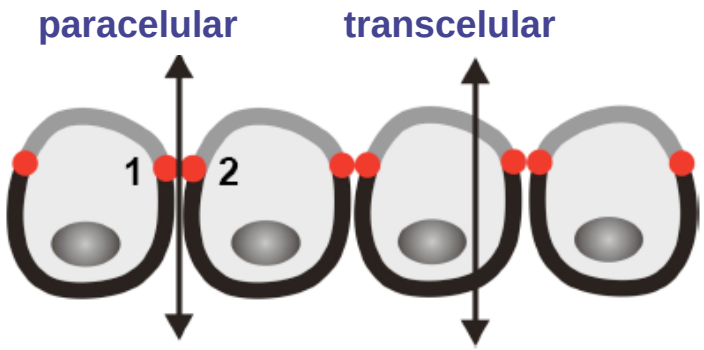
Mechanisms of absorption

- **biological membrane**
  - **coherent bilayer of lipid molecules with outer polar and inner nonpolar part**
  - **inserted proteins working as receptors, enzymes, transport systems, keeping the shape, ...**



Mechanisms of absorption

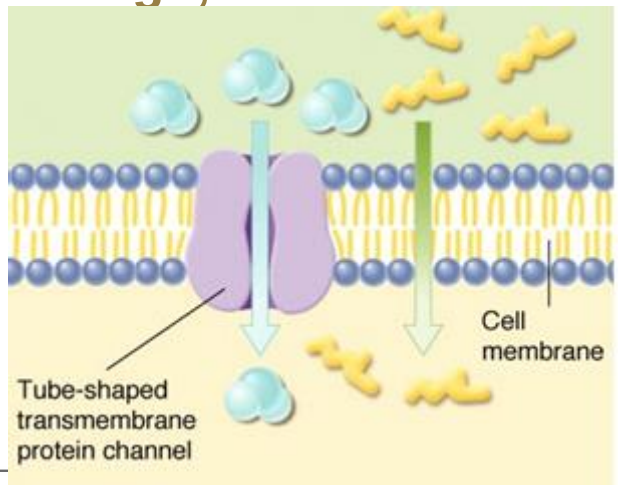
- **paracelular × transcelular**



  - **paracelular**
    - **tight junction**
    - **passive, diffusion (concentracion gradient),**
    - **hydrophilic molecules with low M_r (cimetidin, atenolol)**

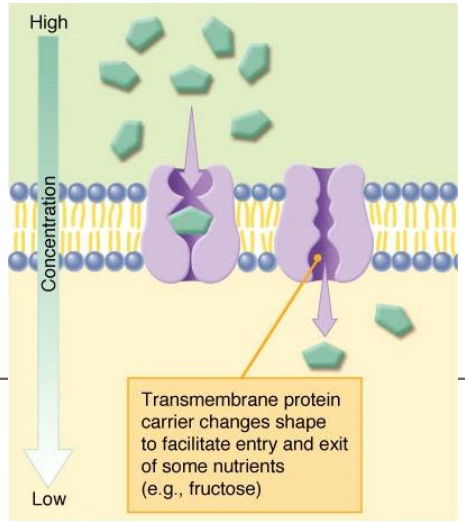
Mechanisms of absorption

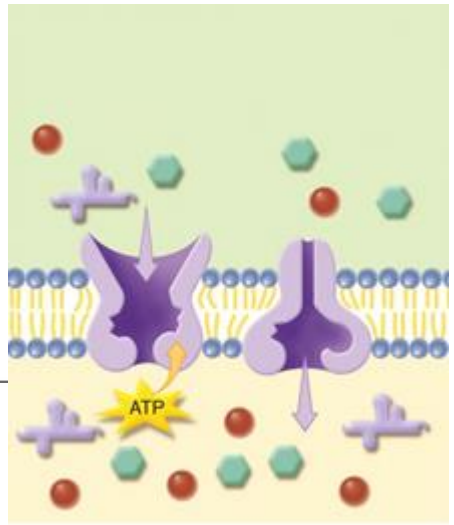
- **transcelular**
  - **lipid diffusion: dissolution in membrane lipids, according to concentration gradient, lipophilic drugs, nonionized form (Henderson-Hasselbalch); majority of drugs**
  - **diffusion across water pores: hydrophilic substances, M_r till 150, low importance for drugs; Li**



Mechanisms of absorption

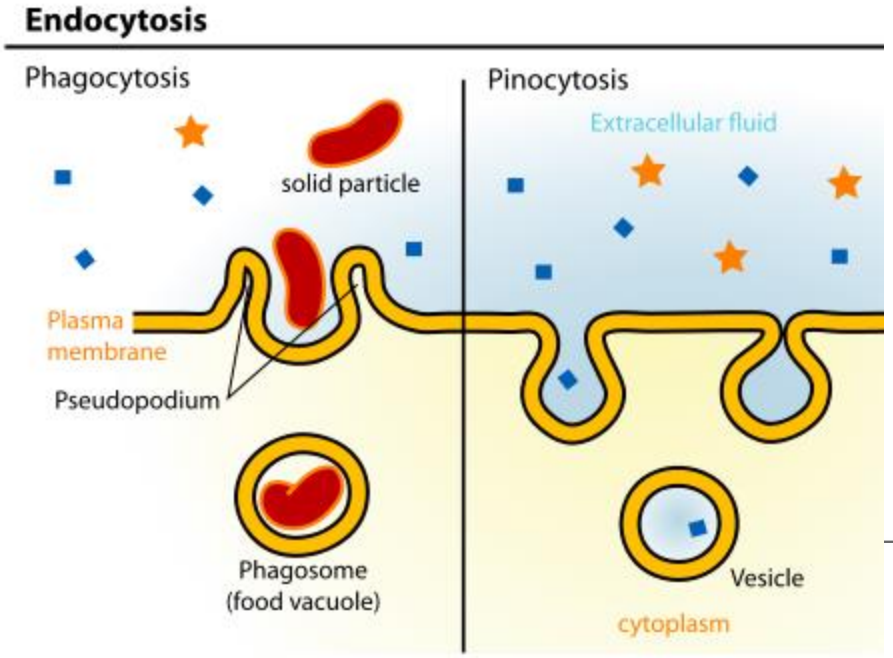
- **transcelular**
  - **facilitated diffusion: with the aid of protein carrier, but only on concentration gradient (protein oscillation); AK-HEB**
  - **active transport: against concentration gradient, energy supply (ATP), with the aid of protein carrier (reversible binding), one-way; levodopa**





Mechanisms of absorption

- **transcelular**
  - **transport on ion pairs: quarter ammonia bases, neutral complex with mucin, pure diffusion**
  - **exocytosis and endocytosis, pinocytosis and fagocytosis**



Absorption influencing factors – Functional status of GIT

- **GIT anatomy**
  - **stomach, small (duodenum, jejunum a ileum) and large intestine**
  - **length and surface of particular parts**
- **transit time**
  - **dissolution, transporters, degradation**
- **local pH**
  - **ionization level, acids, bases**

Absorption influencing factors – Functional status of GIT

- **physiological parameters of GIT**

Part of GIT	Surface (m^2)	Length (m)	Transit time (h)	pH
stomach	0.053	–	0.5 – 1.5*	1.5 – 2
small intestine**	200	6.5 (81 %)	3 – 4	6.0 → 6.5 → 7.0
large intestine	0.35	1.55 (19 %)	8 – 72	6.3

* **transit time in oesophagus: a few seconds**

** **duodenum, jejunum, ileum**

Absorption influencing factors – Functional status of GIT

- **physiological pH in GIT**

Segment	pH	
	fasted	fed
Stomach	1.8 (1 - 3)	4 (3 - 6)
Duodenum	6.0 (4 - 7)	5.0 (4 - 7)
Upper jejunum	6.5 (5.5 - 7)	5.5 (5.5 - 7)
Lower jejunum	6.8 (6 - 7.2)	6.5 (6 - 7.2)
Upper ileum	7.2 (6.5 - 7.5)	7.2 (6.5 - 7.5)
Lower ileum	7.5 (7 - 8)	7.5 (7 - 8)
Proximal colon	5.5 – 6.5	5.5 – 6.5

Absorption influencing factors – Functional status of GIT

- **volume of gastric liquids**
- **local pH (solubility, dissociation)**
- **stomach filling (fasted × fed)**
- **speed of stomach emptying (size of bite, calories amount)**
- **gastrin (absorption increasing)**
- **somatostatin (absorption decreasing)**
- **bile salts (micelles, lipophilic drugs)**

Absorption influencing factors – Functional status of GIT

- **surface area**
- **speed of peristalsis**
- **level of perfusion (congestion)**
- **activity of intestine microflora**
- **activity of digestive enzymes**

- **presystemic elimination: destruction in cells of intestine epithelium, first pass effect, pass through lungs, … !!!**

Usage of absorption models

- **originator companies: to find out, if a candidate has suitable biopharmaceutical properties (peroral absorption – marketing)**
- **generic companies: to find out, if a candidate has a same absorption profile as original product**

Absorption models

- **experiments performed**
  - ***in silico***
  - ***in vitro***
  - ***in situ***
  - ***in vivo***

Experiments performed *in silico*

- **computer models**
  - **on basis of information about a structure and data from preceding *in vivo* experiments, counting of absorption of new substances**
  - **important factors lipophilicity, hydrogen bindings, inter- and intramolecular bindings, charge, molar weight ⇨ prediction of passive transport**
  - **QSAR (Quantitative Structure – Activity Relationship)**

Experiments performed *in vitro*

- **chromatographic methods**
- **artificial lipid membranes**
- **cell cultures**
- **parts of small intestine**
- **(SHIME)**

!!! together with *in silico* methods – saving of animals !!!

Experiments performed *in vitro*

- **chromatographic methods**
  - **stationary phase resembling lipid bilayer or immobilised phospholipids or liposomes**
  - **only interaction with lipid bilayer, not a transportation across a membrane**

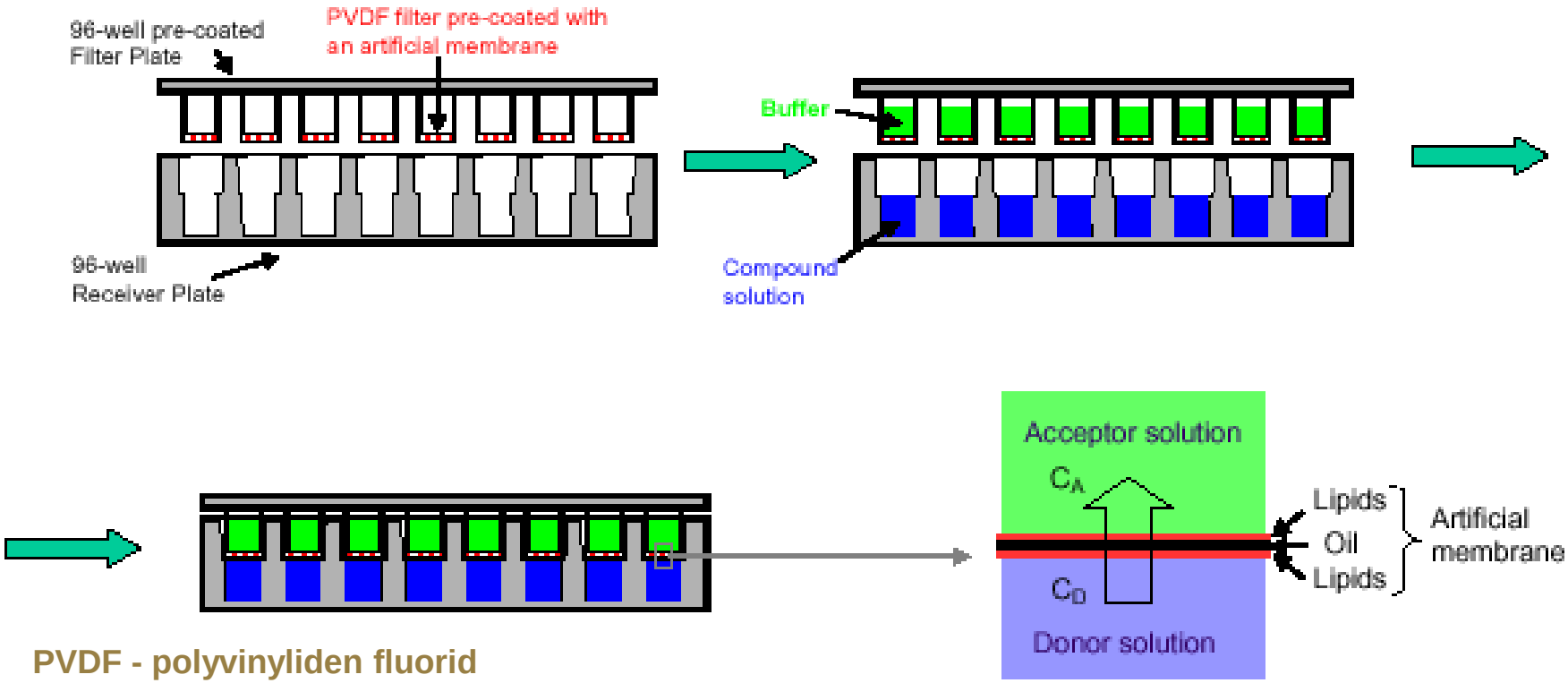
Experiments performed *in vitro*

- **artificial lipid membranes**
  - **hydrophobic porous filter impregnated with phospholipids**
  - **prediction of passive diffusion, transportation across a membrane**
  - **PAMPA (Parallel Artificial Membrane Permeation Assay)**

12 thousand CZK / 5 plates

Experiments performed *in vitro* – PAMPA

➢ PAMPA

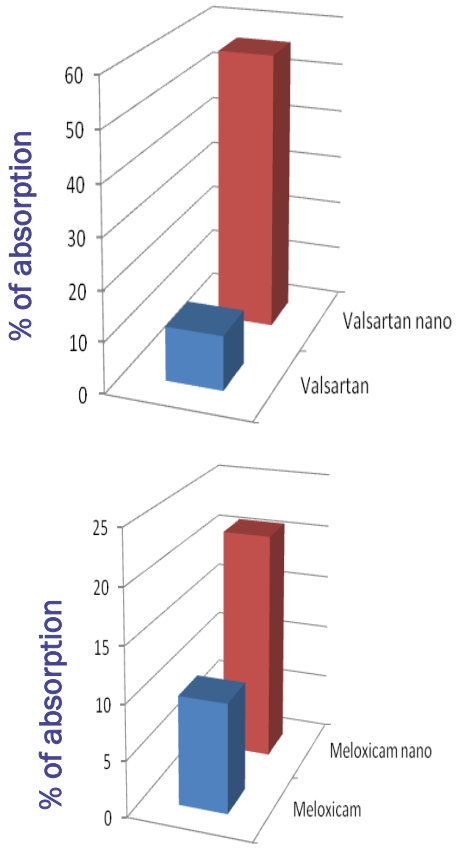


PVDF - polyvinyliden fluorid

Experiments performed *in vitro* – PAMPA

- **comparison of nanonized and standard API**

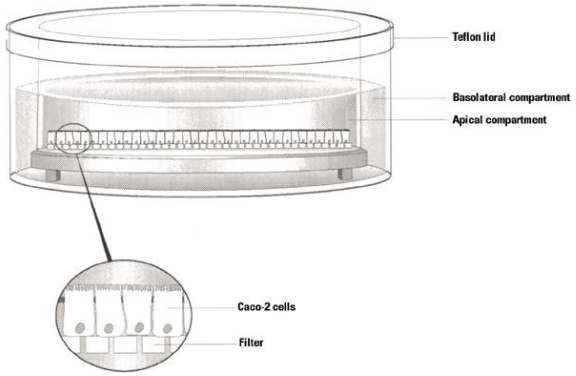
API	Particle size (nm) (Nano API (x_{90}))	Particle size (µm) (standard API)
Meloxicam	290	20
Valsartan	697	30
API	Permeability (%) (Nano API)	Permeability (%) (standard API)
Meloxicam	20.8	9.8
Valsartan	56.0	10.7



Experiments performed *in vitro*

- **cell cultures**
  - **prediction of transportation across a cell membrane including active transport**
  - **Caco-2 cells** **10 500 – 22 500 CZK / 1 compound**

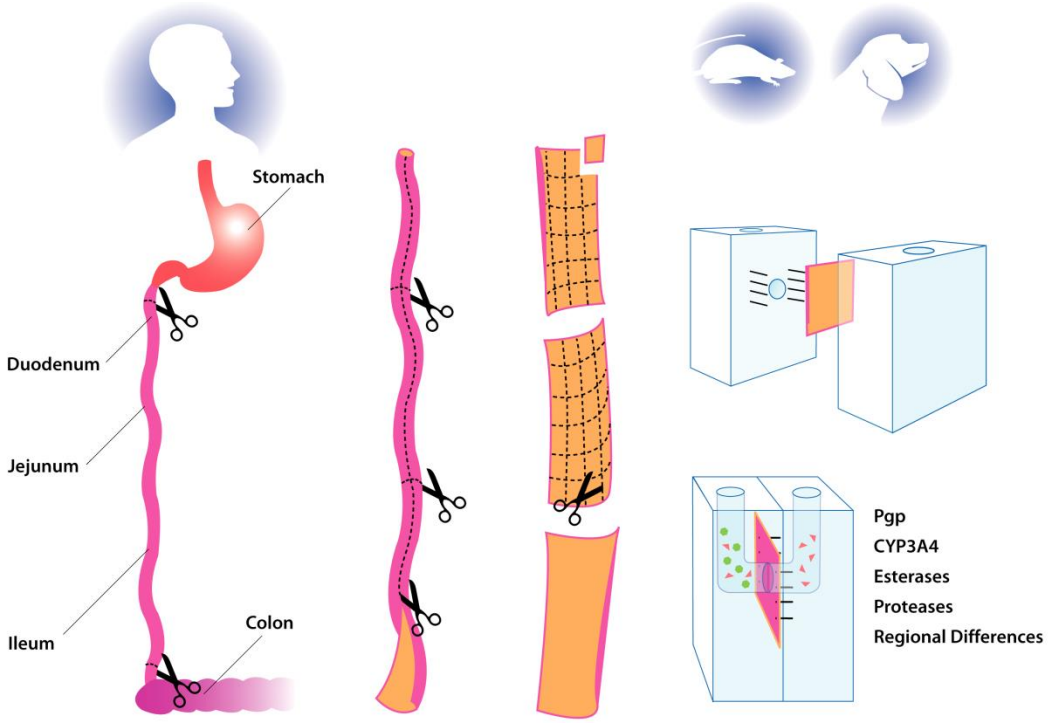
Experiments performed *in vitro*



- **Caco-2 cells**
  - **cells of human adenocarcinoma of large intestine (colon)**
  - **suggested for simulation and prediction of intestinal drug absorption after oral administration**
  - **dispose of enzymatic and transportation systems (with limitations – low capacity)**
  - **correlation with *in vivo* data has shown that Caco-2 cells are able to predict absorption *in vivo* and identify low permeable substances; BCS classification**
  - **low amount of tested compound**
  - **not possible to distinguish differences in absorption in particular parts of small intestine, better for APIs than drug forms, narrow range of working pH, time consuming**

Experiments performed *in vitro*

- **parts of small intestine**
  - **human**
  - **rat, dog**
  - **Ussing chamber**
  - **absorption in particular parts**

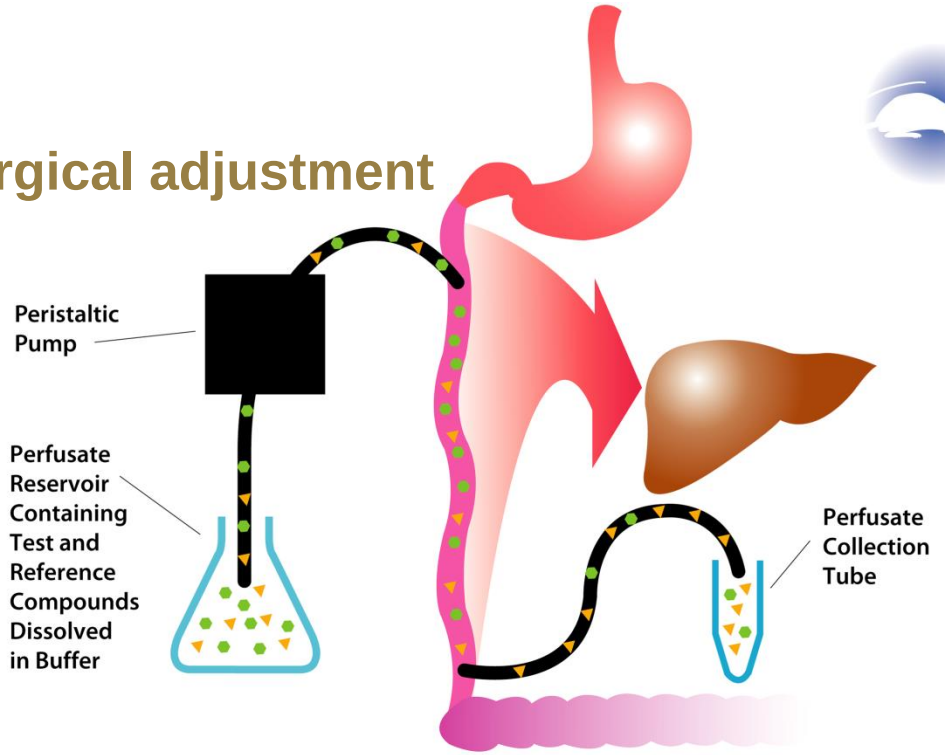


Experiments performed *in vitro*

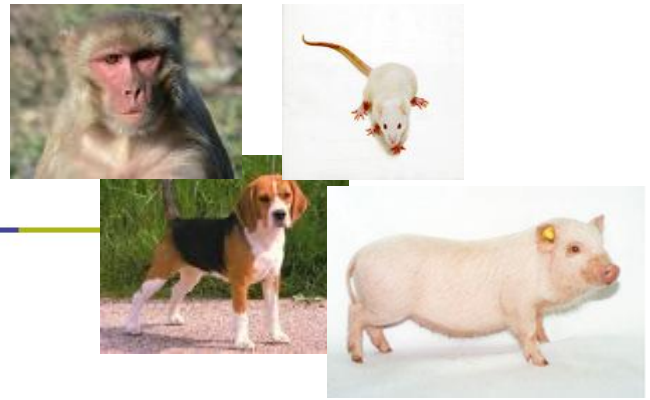
- **SHIME (Simulation of the Human Intestinal Microbial System):**
  - **model, which simulates physicochemical and enzymatic conditions and contains bacterial colonies resembling colonies situated in human GIT**
  - **multi-compartment 5-step system, 2 steps small intestine, 3 steps large intestine**
  - **decomposition of active molecule × prodrug (Sulfasalazin)**

Experiments performed *in situ*

- **perfused organs**
  - **comparison of isolated absorption**
  - **solution, ~~solid dosage forms~~**
  - **mass balance**
  - **small sample series – surgical adjustment**
  - **anaesthesia**



Experiments performed *in vivo*



- **animal models**
  - **mouse**
  - **rat**
  - **guinea pig**
  - **dog**
  - **pig**
  - **mini-pig**
  - **ape**
  - **surgical adjusted (probes etc.)**
  - **there is not a single universal suitable mate of animal model**

6 000 USD / 1 compound

Thank you for your attention