Physico-chemical properties of compounds

MBDD 22.2.2018

ADME :
Absorption – **D**istribution – **M**etabolism – **E**xcretion

Exploration of ADME is at least of same importance as exploration of activity of the compound.

Large pharmaceutical companies are able to screen over **3 000 000** of new molecules for biological activity per year.
Some **30 000** hits may be found.
Most of them, however potent they are, have not suitable physical, metabolic and safety properties.
Some **30** molecules pass for pharmacological evaluation.
0 - 3 molecules are introduced into market.
About 30% of molecules in pharmacological evaluation are rejected due to ADME problems.

„**A**“ from **ADME** (Absorption)

properties affecting passive absorption:
acid-base character
lipophilicity
solubility
membrane permeability

Transport model

permeability – solubility - charge state – pH-partition hypothesis

Passive diffusion: product of diffusivity and concentration gradient

For ionizable molecules to permeate, molecule needs to be uncharged

The amount of uncharged form is pH-dependent

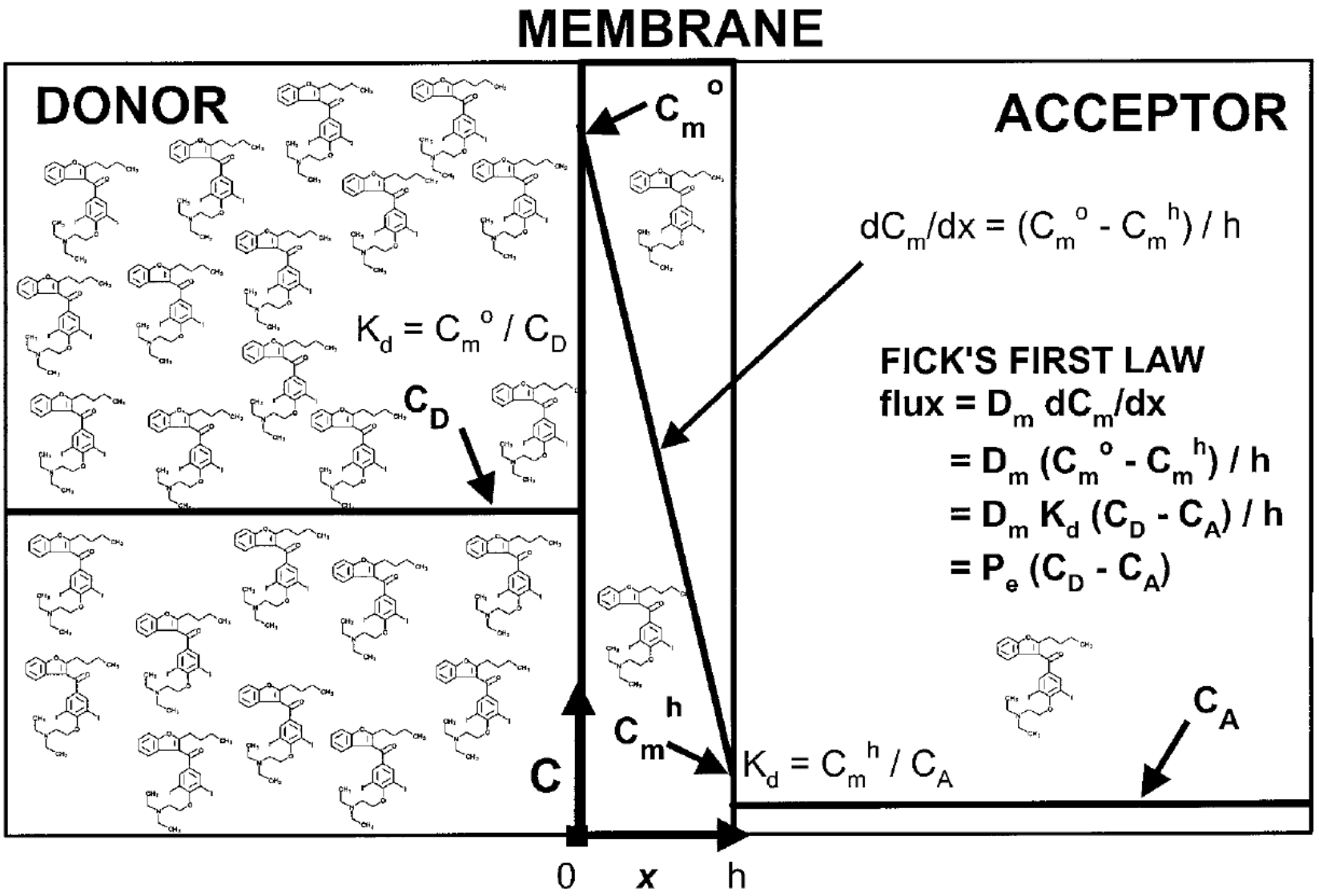


Figure 2.1 Transport model diagram, depicting two aqueous cells separated by a membrane barrier. The drug molecules are introduced in the donor cell. The concentration gradient in the membrane drives the molecules in the direction of the acceptor compartment. The apparent partition coefficient, $K_d = 2$. [Avdeef, A., *Curr. Topics Med. Chem.*, **1**, 277–351 (2001). Reproduced with permission from Bentham Science Publishers, Ltd.]

C_m^0, C_m^h : concentrations of uncharged forms in membrane (hard to estimate)

h : thickness of membrane

logK : partition coefficient lipid/water

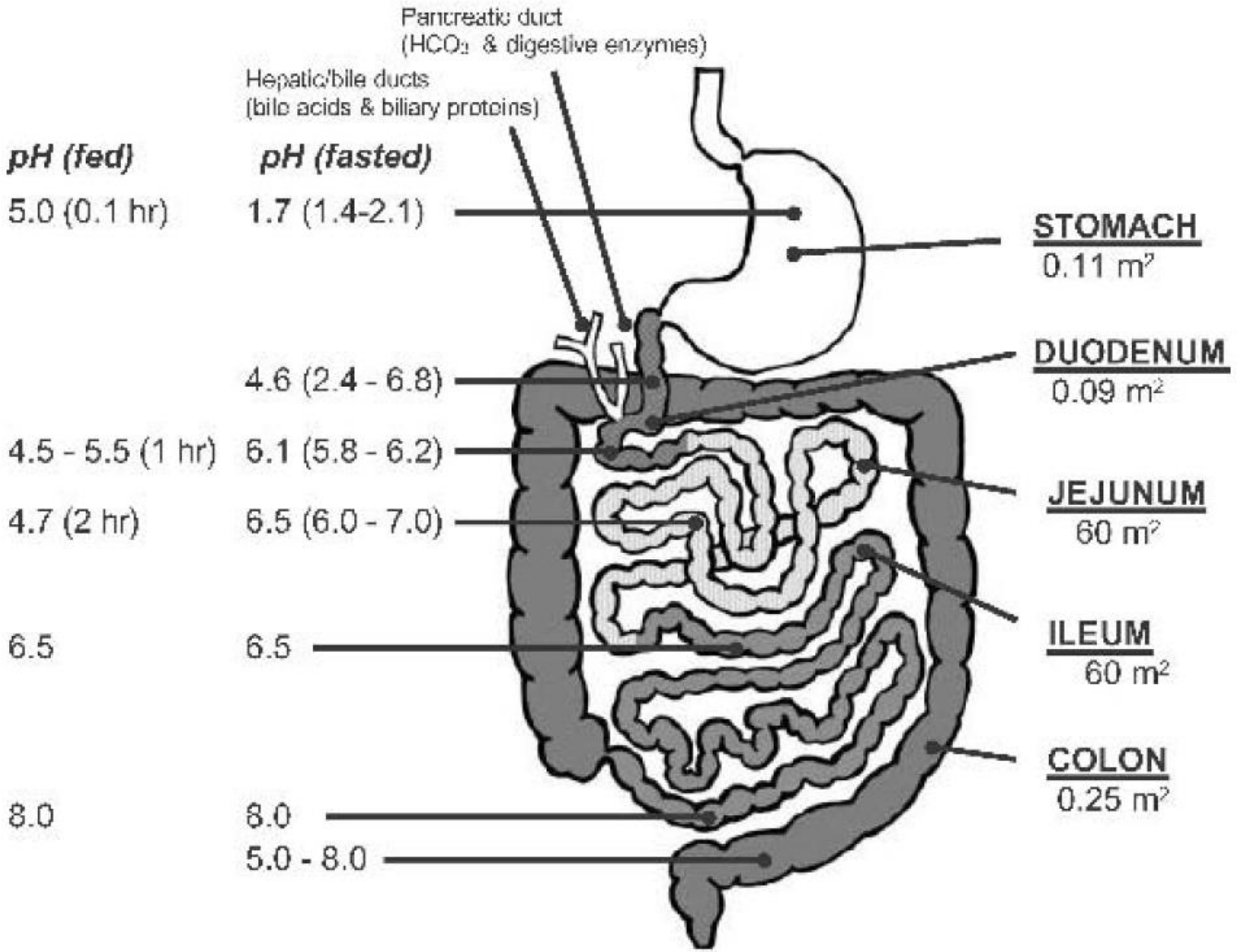
C_D, C_A : water concentrations (easy to estimate by HPLC)

D_m : diffusivity

P_m : permeability

$$P_m = \frac{D_m K_d}{h}$$

Physiological properties of the GIT



Jejunum + ileum > 99% of absorbable surface

Time needed to pass through:

Empty stomach : water solution up to 0.4 hour

solids 0.5 - 3 hours

fatty food up to 13 hours

Jejunum + ileum : 3 – 5 hours

Colon: 7 – 20 hours (depends of sleeping period)

pH decrease in colon may be caused by short fatty acids produced by bacteria

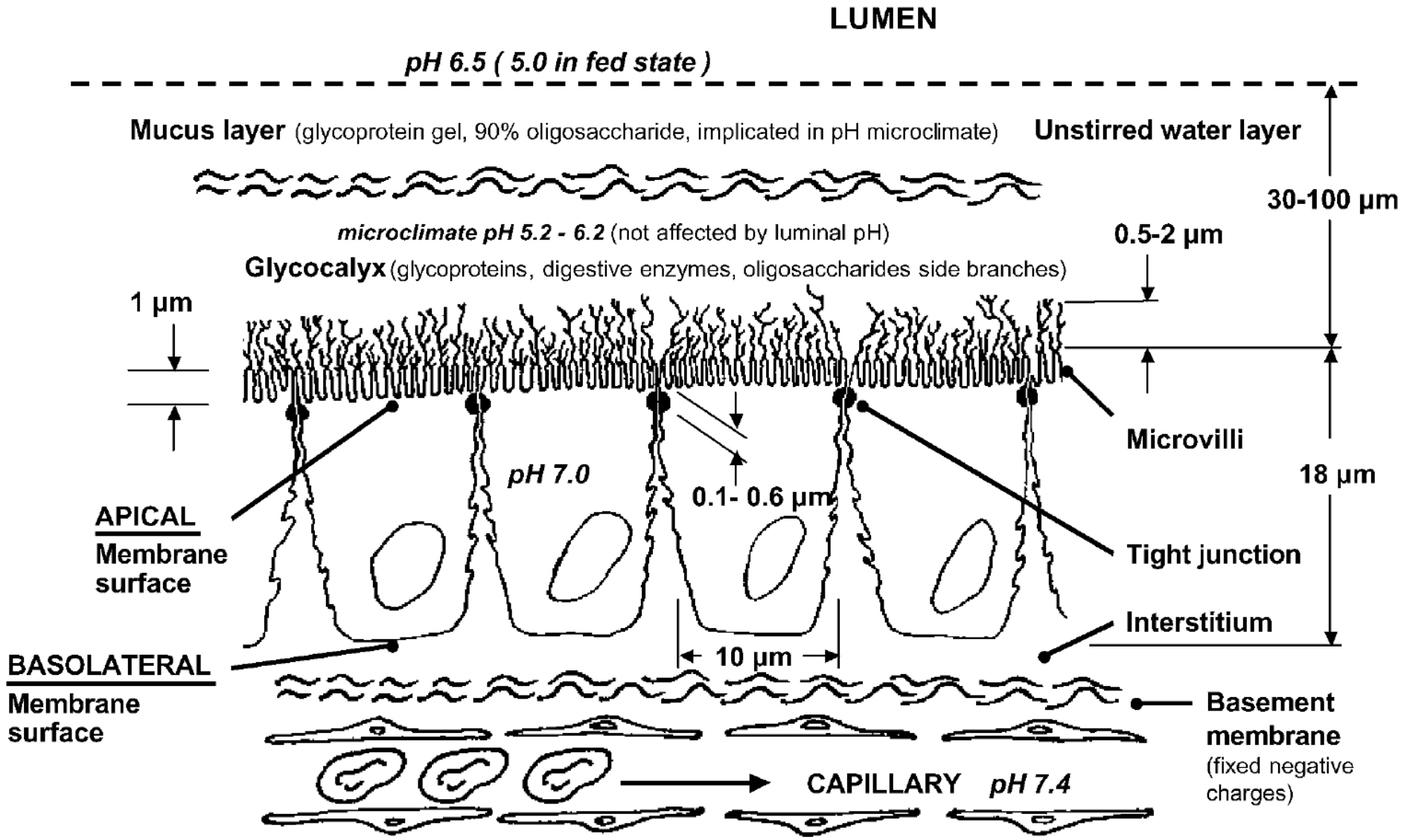


Figure 2.5 Schematic of the structure of epithelial cells, based on several literature sources [55,63,69,73,74,76,78,79]. The tight junctions and the basement membrane appear to be slightly ion-selective (lined with some negatively charged groups) [75,76,79]. [Avdeef, A., *Curr. Topics Med. Chem.*, **1**, 277–351 (2001). Reproduced with permission from Bentham Science Publishers, Ltd.]

-glycocalyx slows absorption of lipophillic molecules

-tight junctions allows to come through small molecules (< 200 Da)

-positively charged drugs has better permeability through basal membrane

-acid pH microclimate prefer weak acids for permeation

Intracellular pH environment:

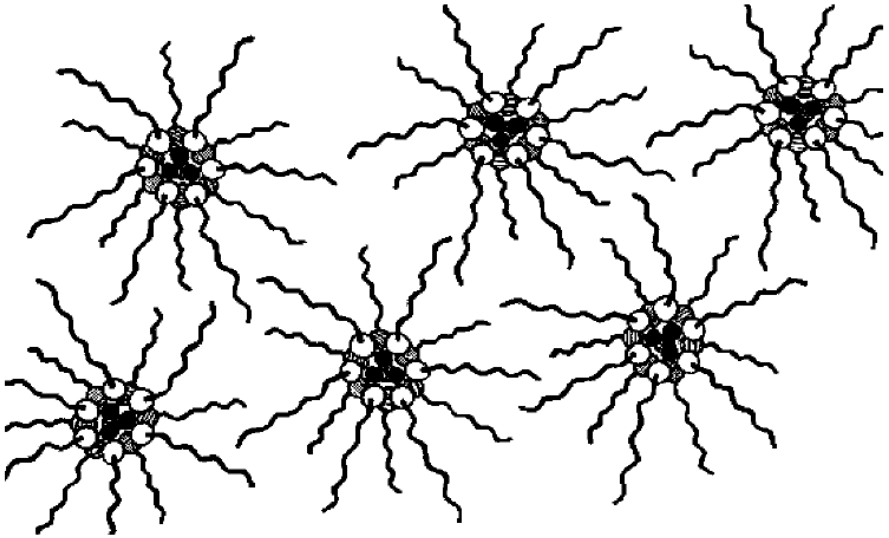
TABLE 2.1 Intracellular pH Environment

Intracellular Compartment	pH
Mitocondria	8.0
Cytosol	7.2–7.4
Endoplasmic reticulum	7.1–7.2
Golgi	6.2–7.0
Endosomes	5.5–6.0
Secretory granules	5.0–6.0
Lysosomes	4.5–5.0

Structure of octanol.

Octanol serves for years as a model.

Water saturated octanol:



water-octanol clusters allows to enter hydrophyllic compounds, partially charged compounds, as well

Biopharmaceutics classification system

Four BCS classes due to solubility and permeability

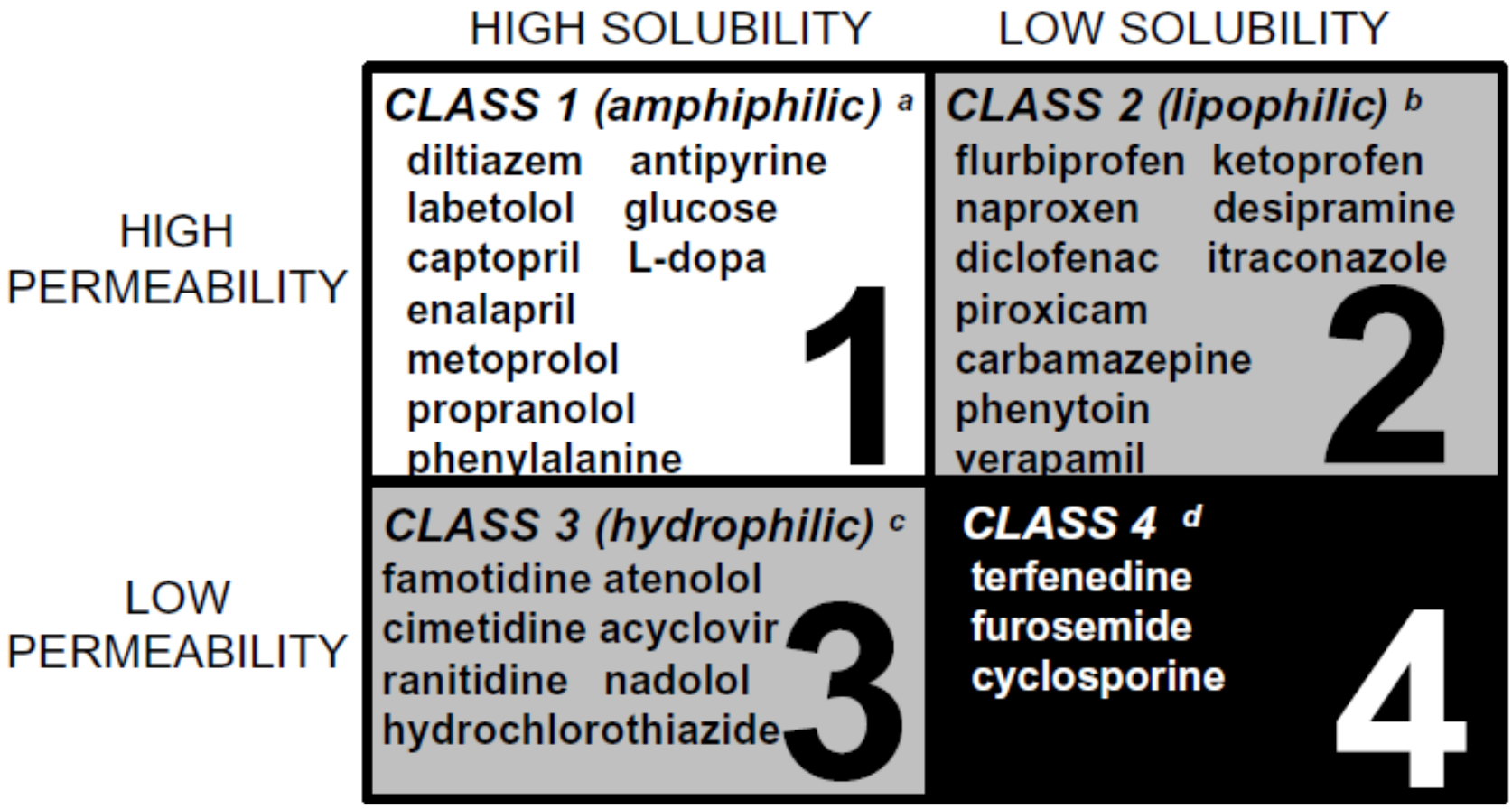
For this classification:

Solubility is amount of watter needed to dissolve highest single dose at pH (1-8) with worst solubility: low s > 250 mL > high s

Permeability in human jejunum in vivo

high > 10^{-4} > low

Biopharmaceutics classification system



Charge state

Weak acids and bases ionize in solutions to varying extent, dependent on pH of environment. This affects amount of uncharged molecules ready for absorption.

Thermodynamic parameter of this process is the ionization constant K_A (pK_A)

Knowlwdge of compound´s pK_A is very imortant – it can predict absorption, distribution and excretion of the compound.

Charge state Example

Urine pH (normal 5.7-5.8) can be altered by oral doses of NH_4Cl or $NaHCO_3$ to ease excretion of ionized compounds in toxicological emergencies. Weak acids are excreted in alkaline urine, weak bases in acidic urine.

Henderson-Hasselbach equation

thermodynamic equations for acid, base and diprotic ampholyte:

$$\mathrm{HA} \rightleftarrows \mathrm{A^-} + \mathrm{H^+} \qquad K_a = \frac{[\mathrm{A^-}][\mathrm{H^+}]}{[\mathrm{HA}]} \qquad (3.1)$$

$$\mathrm{BH^+} \rightleftarrows \mathrm{B} + \mathrm{H^+} \qquad K_a = \frac{[\mathrm{B}][\mathrm{H^+}]}{[\mathrm{BH^+}]} \qquad (3.2)$$

$$\mathrm{XH_2^+} \rightleftarrows \mathrm{XH} + \mathrm{H^+} \qquad K_{a1} = \frac{[\mathrm{XH}][\mathrm{H^+}]}{[\mathrm{XH_2^+}]} \qquad (3.3)$$

$$\mathrm{XH} \rightleftarrows \mathrm{X^-} + \mathrm{H^+} \qquad K_{a2} = \frac{[\mathrm{X^-}][\mathrm{H^+}]}{[\mathrm{XH}]} \qquad (3.4)$$

negative logarithm of these equations give Henderson-Hasselbach equations:

Henderson-Hasselbach equation

$$pK_a = pH + \log \frac{[HA]}{[A^-]} \quad (3.5)$$

$$pK_a = pH + \log \frac{[BH^+]}{[B]} \quad (3.6)$$

$$pK_{a1} = pH + \log \frac{[XH_2^+]}{[XH]} \quad (3.7)$$

$$pK_{a2} = pH + \log \frac{[XH]}{[X^-]} \quad (3.8)$$

$pH = pK_A$: concentration of ionized and uncharged form is equal

$pH = pK_A - 2$: ratio (1:100) 99.9% uncharged

$pH = pK_A + 2$: ratio (100:1) 99.9% charged

Constant ionic medium reference state

Ionic strenght of the solution is involved in dissociation rates.

Measurements of pK_A has to be performed in standard ionic conditions:

0.15 M KCl or NaCl solutions are used (physiological ionic strenght)

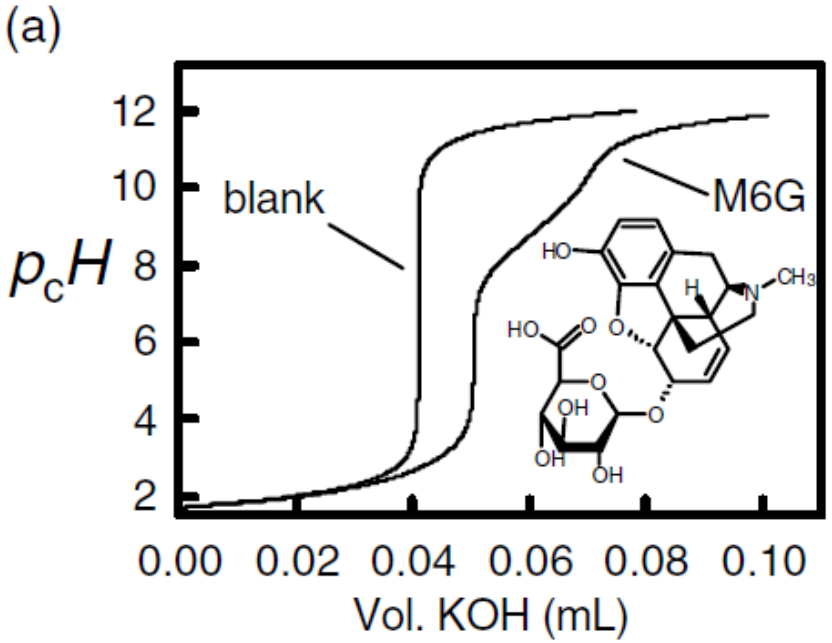
Potentiometric measurement

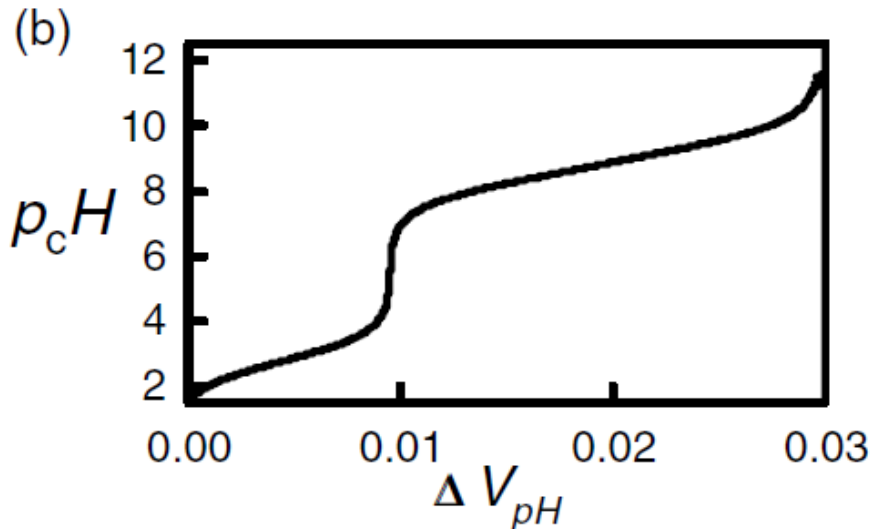
Titration of water solutions of substance with addition of 0.15 M KCl or NaCl
by HCl or KOH/NaOH
Potentiometric titration curve is obtained

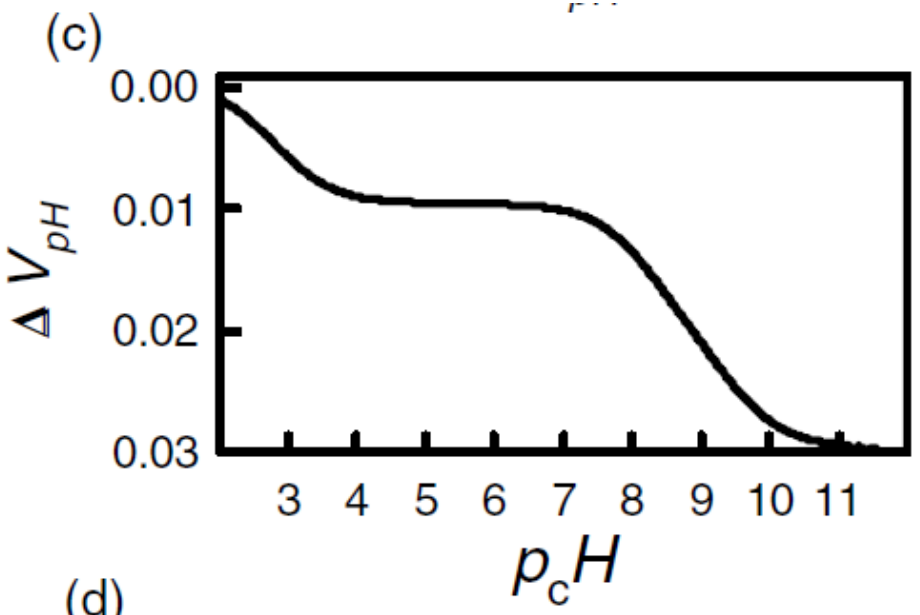
In the case of multiprotic compounds, simple curve can be misleading!

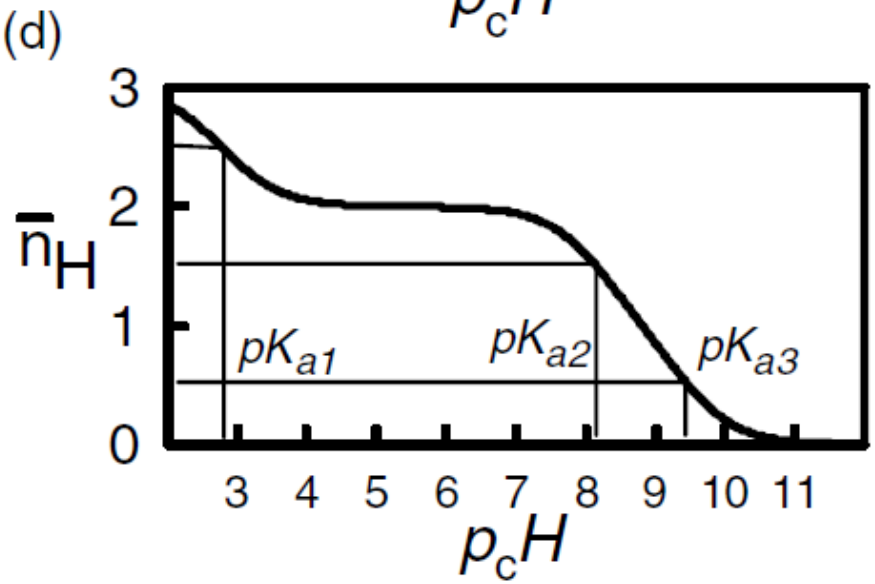
Bjerrum plot must be constructed:

Bjerrum plot construction:









Bjerrum plot construction:

1. Subtract titration curve with no compound (blank) from a titration curve with sample (b)
2. x and y axis are rotated (c)
3. volume difference is turned to number of ionizable hydrogens ratio (known from structure): Difference between total ionizable hydrogens and actually ionized hydrogens

Equilibrium states indicates pK_A values (d)

Solubility problems:

Most of bioactive compounds are poorly soluble in water.

> 100 µM: no problems in potentiometry

10 – 100 µM: measurable after carefull electrode calibration

< 10 µM: mixed solvent environment have to be used

Co-solvent mixtures:

alcohol - water (methanol, ethanol, propanol)
dimethylsulfoxide (DMSO) – water
dioxane – water

Measured values in the mixture can be extrapolated to poor water using standard sets

Spectroscopic measurement (UV-VIS):
pH-dependent chromophore necessary

construction of molar absorbance to pH curves
at various vawelenghts
searching for suitable vawelenghts

specific method development for each compound
needed

Capillary electrophoresis mesurements

mobility of ionizable compounds depends on pK_A

apparent mobility to pH curves are constructed

sigmoideal shape, midpoint pH equals to pK_A

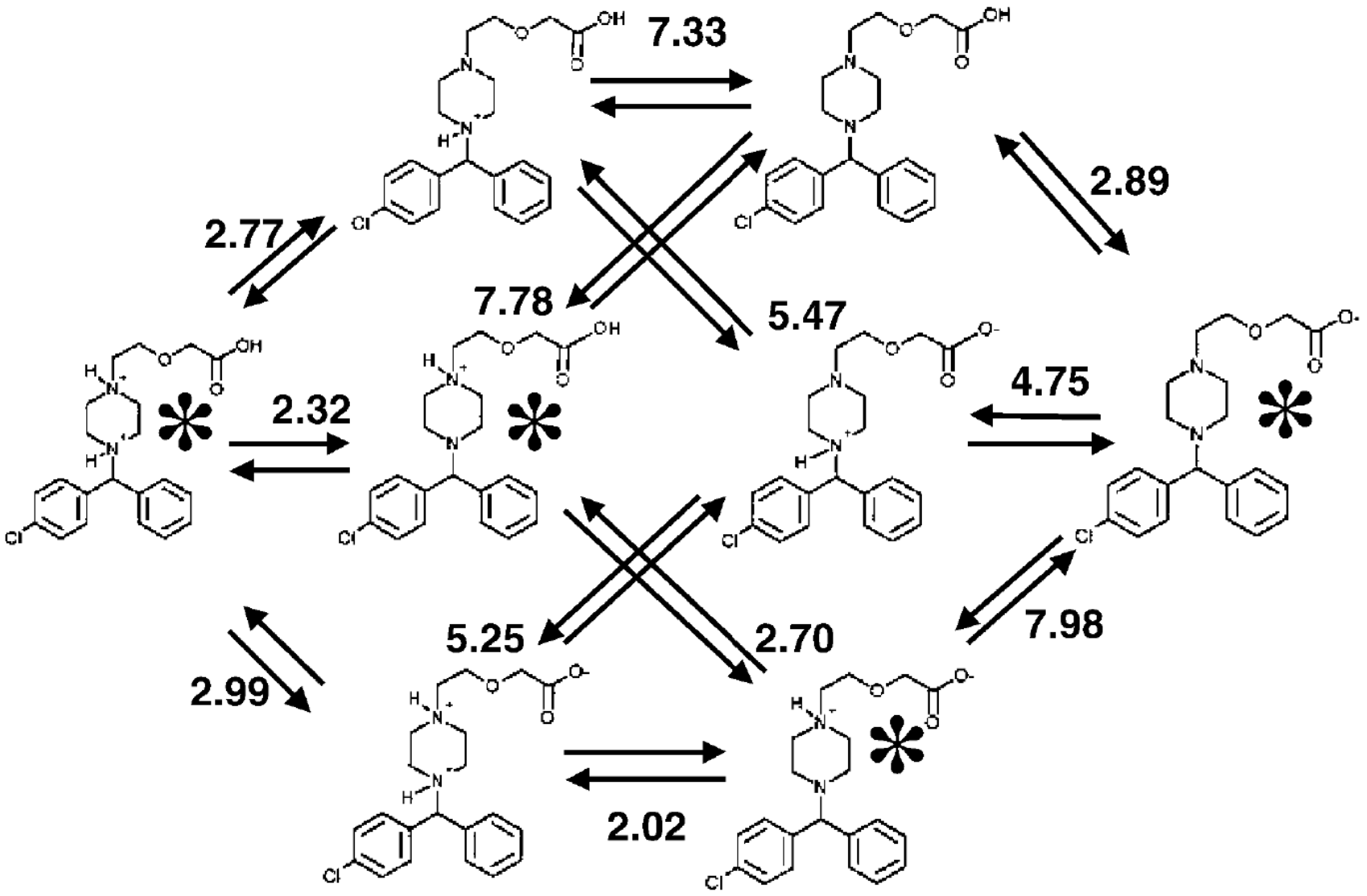
Macroconstants / microconstants

Certain type of multiprotic molecules posses different tautomeric arrangement

measured pK_A are average constants for more complex equilibria – macroconstants

Microconstants can be elucidated by multiple series of measurements with cosolvents shifting pKa values in combination with UV-VIS detection of chromophore changing

Macroconstants / microconstants (cetirizine)



Partitioning into octanol

P – partition coefficient

D – apparent partition coefficient (pH-dependent)

Partitioning equilibria:

$$\mathrm{HA} \rightleftarrows \mathrm{HA}_{(\mathrm{ORG})} \qquad (\mathrm{B} \rightleftarrows \mathrm{B}_{(\mathrm{ORG})})$$

$$P_{\mathrm{HA}} = \frac{[\mathrm{HA}_{(\mathrm{ORG})}]}{[\mathrm{HA}]} \qquad \left(P_{\mathrm{B}} = \frac{[\mathrm{B}_{(\mathrm{ORG})}]}{[\mathrm{B}]}\right)$$

non-ionizable molecules can be directly measured

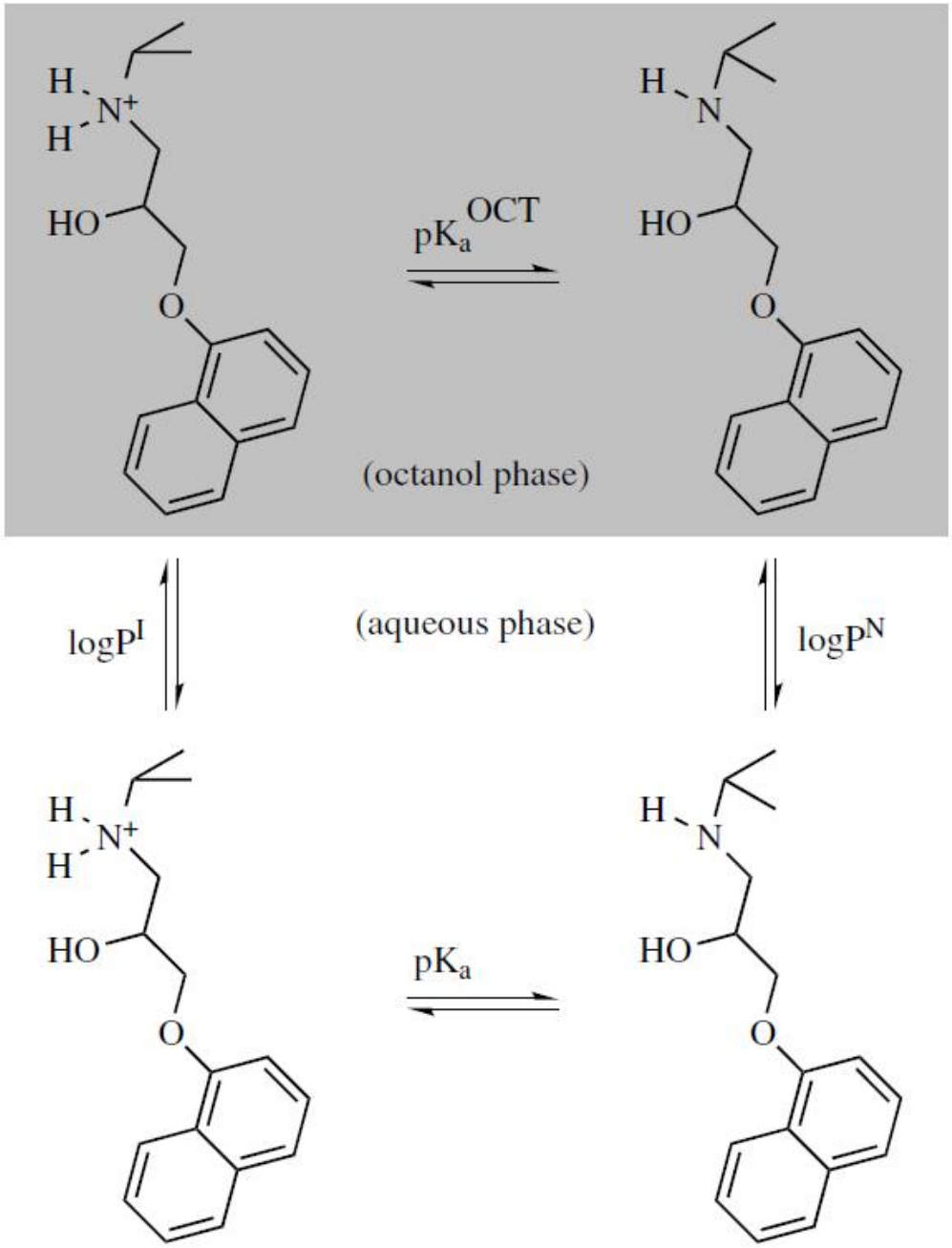
Partitioning into octanol

ionizable molecules are partitioned too, but to much lesser extent:

$$A^- \rightleftarrows A^-_{(ORG)} \qquad (BH^+ \rightleftarrows BH^+_{(ORG)})$$

$$P_A = \frac{\left[A^-_{(ORG)}\right]}{[A^-]} \qquad \left(P_{BH} = \frac{\left[BH^+_{(ORG)}\right]}{[BH^+]}\right)$$

Partitioning into octanol:
Propranolol



Partitioning into octanol

$$diff\,(\log P^{N-I}) = \log P^{N} - \log P^{I} = \left|\mathrm{p}K_a^{\mathrm{oct}} - \mathrm{p}K_a\right|$$

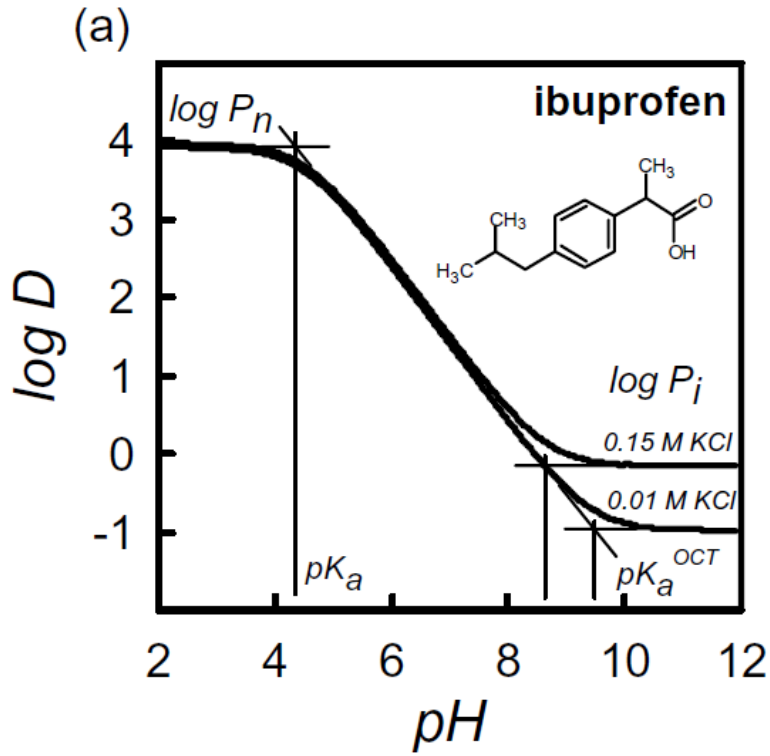
Difference between ionized and non-ionized forms:
Difference between partition coefficients is equal to difference between pKas

logD

Distribution ratio D is used at ionizable molecules refers to a collection of all species:

$$D = \frac{([X_{(ORG)}]' + [XH_{(ORG)}]' + [XH_{2(ORG)}]' + \cdots)/([X] + [XH] + [XH_2] + \cdots)}{r}$$

lipophilicity profiles:



Shake-flask method

concentration is determined in aqueous phase (HPLC)

HPLC methods

retention times at hydrophobic column/aqueous buffer system are hydrophobicity indices

logP values can be calculated from retention times using standards (structurally similar compounds with known logP)

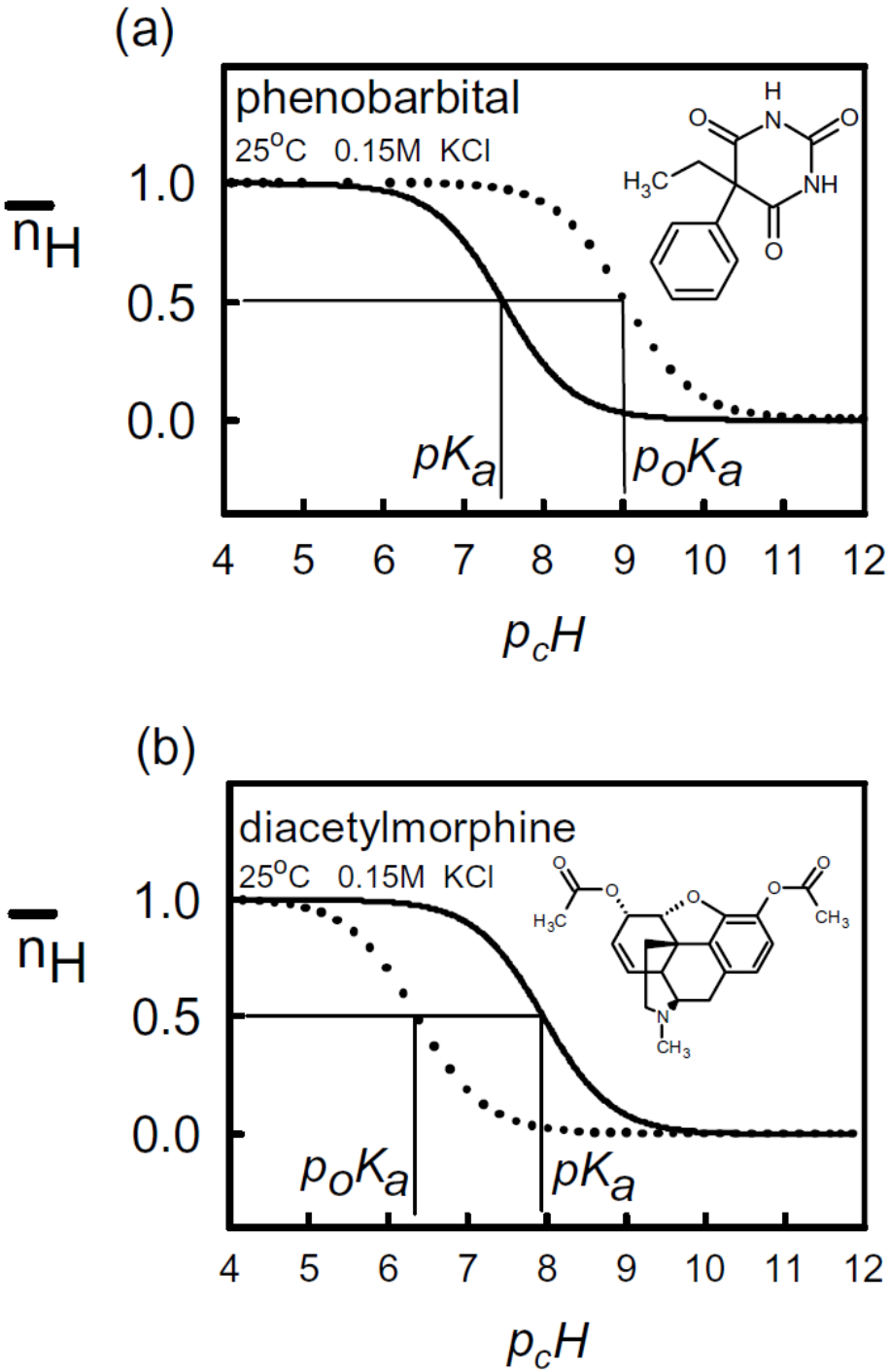
pH metric logP method

dual phase titration of compounds

Bjerrum plots are constructed to potentiometric curves for octanol and water separately

Difference between pKa and apparent pKa is equal to partition coefficient

pH metric logP method

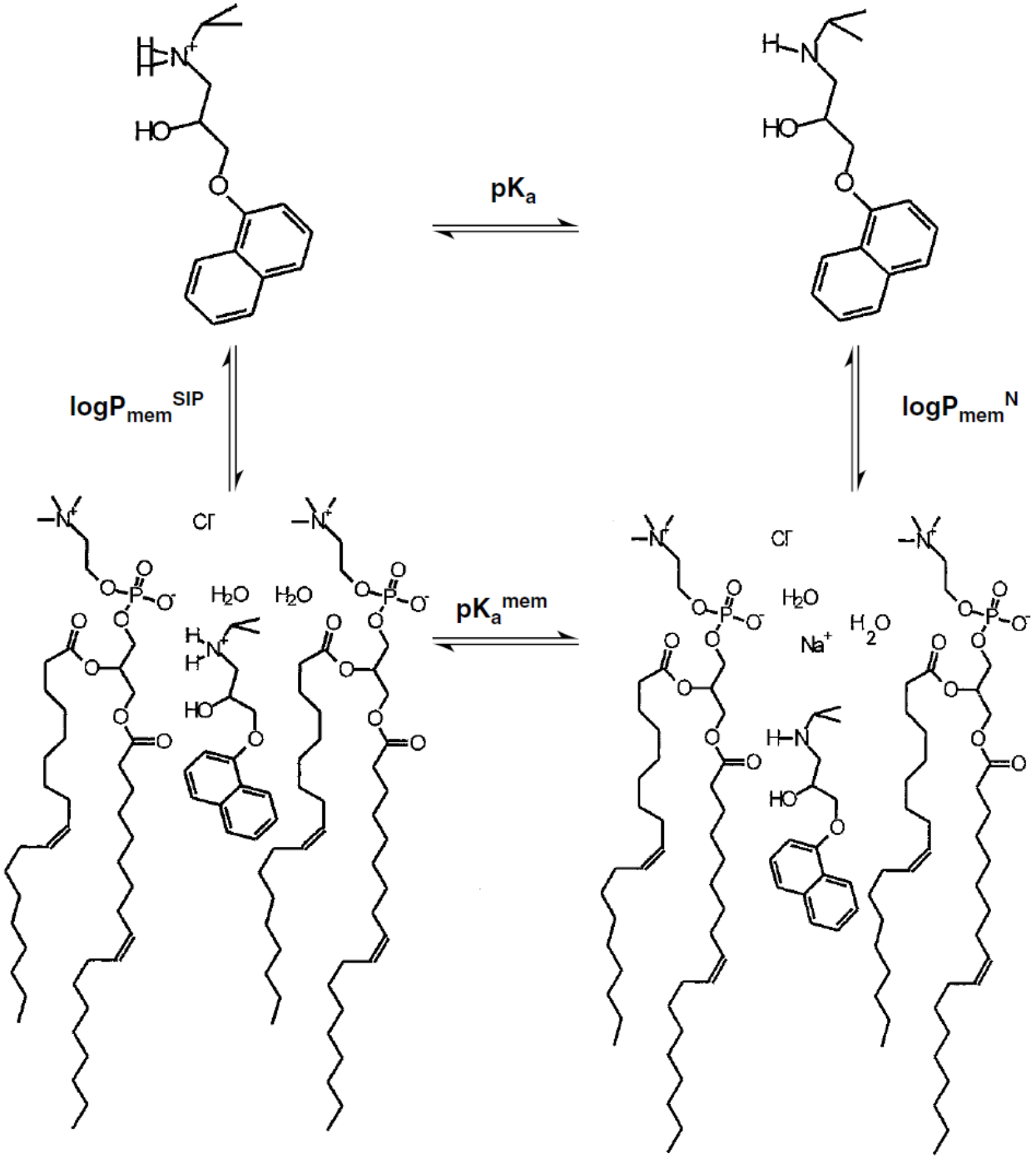


Partitioning into liposomes

liposomes are more „biologic-like“ distribution between membrane and aqueous phase

$$BH^{+}_{(mem)} \rightleftarrows B_{(mem)} + H^{+} \qquad K_a^{mem} = \frac{[B_{(mem)}][H^{+}]}{[BH^{+}_{(mem)}]}$$

Partitioning into liposomes

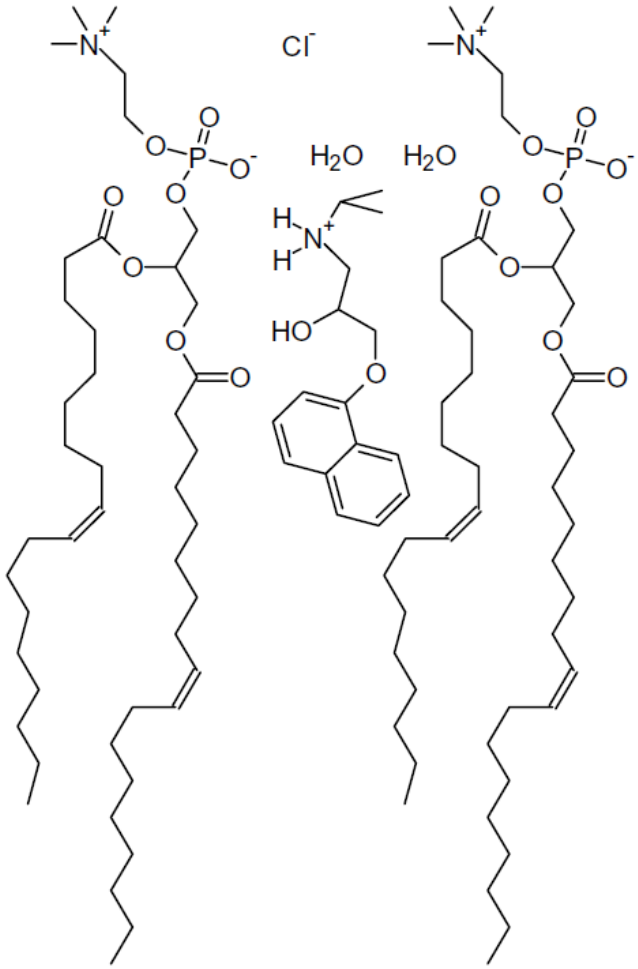


Partitioning into liposomes

Changing of dielectric properties of a layer

BULK WATER

78 (I=0)
75 (I=0.15M)



40-50
(positive electrostatics)

34 (I=0.03M)
29 (I=0.5M)
(negative electrostatics)

20-25
(carbonyl H-bond acceptors)

10
(cis double bonds)

2
(hydrocarbon core)

Partitioning into liposomes

Liposomes are added to defined solution of the compound

after equilibria is established, liposomes are separated by:

dialysis

ultrafiltration

centrifugation

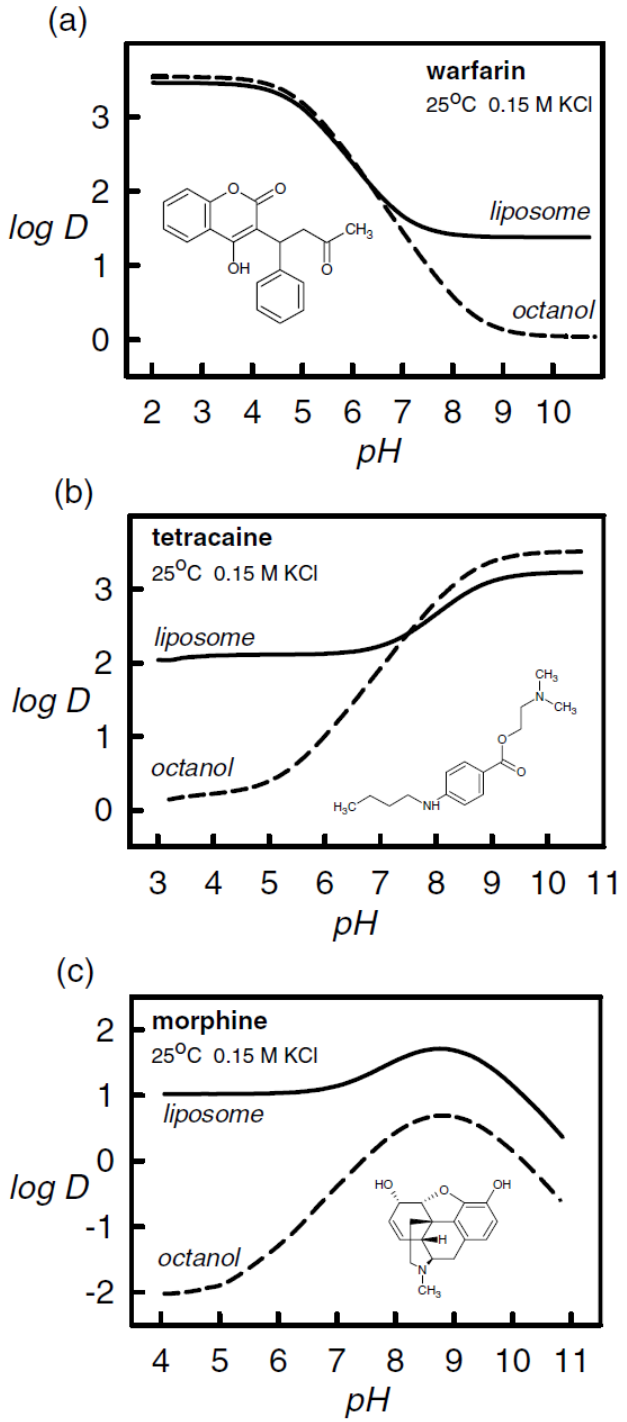
Amount of unabsorbed substance in water solution is determined

Partitioning into liposomes

liposome partitioning can be estimated from logP valuaes by complex computational process

Partitioning into liposomes

complex non-linear similarity



Solubility

solubility of ionizable molecules depends on pKa

monoprotic molecules:

$$S = [\mathrm{A}^-] + [\mathrm{HA}] \qquad [\text{or } S = [\mathrm{B}] + [\mathrm{BH}^+]]$$

$$S = \frac{[\mathrm{HA}]\,K_\mathrm{a}}{[\mathrm{H}^+]} + [\mathrm{HA}] \qquad \left(\text{or } S = [\mathrm{B}] + \frac{[\mathrm{B}][\mathrm{H}^+]}{K_\mathrm{a}}\right)$$

$$= [\mathrm{HA}]\left(\frac{K_\mathrm{a}}{[\mathrm{H}^+]} + 1\right) \qquad \left(\text{or } = [\mathrm{B}]\left\{\frac{[\mathrm{H}^+]}{K_\mathrm{a}} + 1\right\}\right)$$

$$= S_0(10^{-\mathrm{p}K_a + \mathrm{pH}} + 1) \qquad (\text{or } = S_0\{10^{+\mathrm{p}K_a - \mathrm{pH}} + 1\})$$

diprotic ampholyte:

$$S = [\mathrm{X}^-] + [\mathrm{XH}] + [\mathrm{XH}_2^+]$$

$$S = S_0(1 + 10^{-\mathrm{p}K_{a2} + \mathrm{pH}} + 10^{+\mathrm{p}K_{a1} - \mathrm{pH}})$$

Solubility

many experimental complications:

crystalline/amorphous form

amorphism

polymorphism

solvates of solids

crystalline cosolvent

self-associates formation

micelles formation

Shake-flask method

thermostated saturated solution is shaken between two phases (solid/liquid)

long equlibrium times (12hours – 7 days)

concentration in water phase is determined by HPLC after microfiltration and centrifugation

Membrane permeability

In simple model, permeability can be linearly related to the membrane-water partition coefficient

In practice, unlinearity often occures:

-unstirred water layer

-aqueous pores in membranes

-membrane retention of lipophilic solute

-precipitation of solute

-transmembrane pH gradients

-hydrogen-bonding, electrostatic, hydrophobic interactions with membrane constituents

-membrane surface charge

Membrane permeability

in vivo additional problems:

-different composition of inner and outer surface

-active transporters

-efflux system P-gp

-metabolism in membrane

Artificial membrane models

Paralell artificial-membrane permeability assay (PAMPA)

-sendwich microplates covered by phospholipide bilayer

-composition near to cell membrane

-allows high-throughoutput screening

Cell monolayer models

permeability through epitelial cell monolayer

e. g. caco-2 cell line