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Pharma Algorithms

# **Ionization-Specific Prediction of Passive Permeability of Drugs Across Caco-2 Monolayers**

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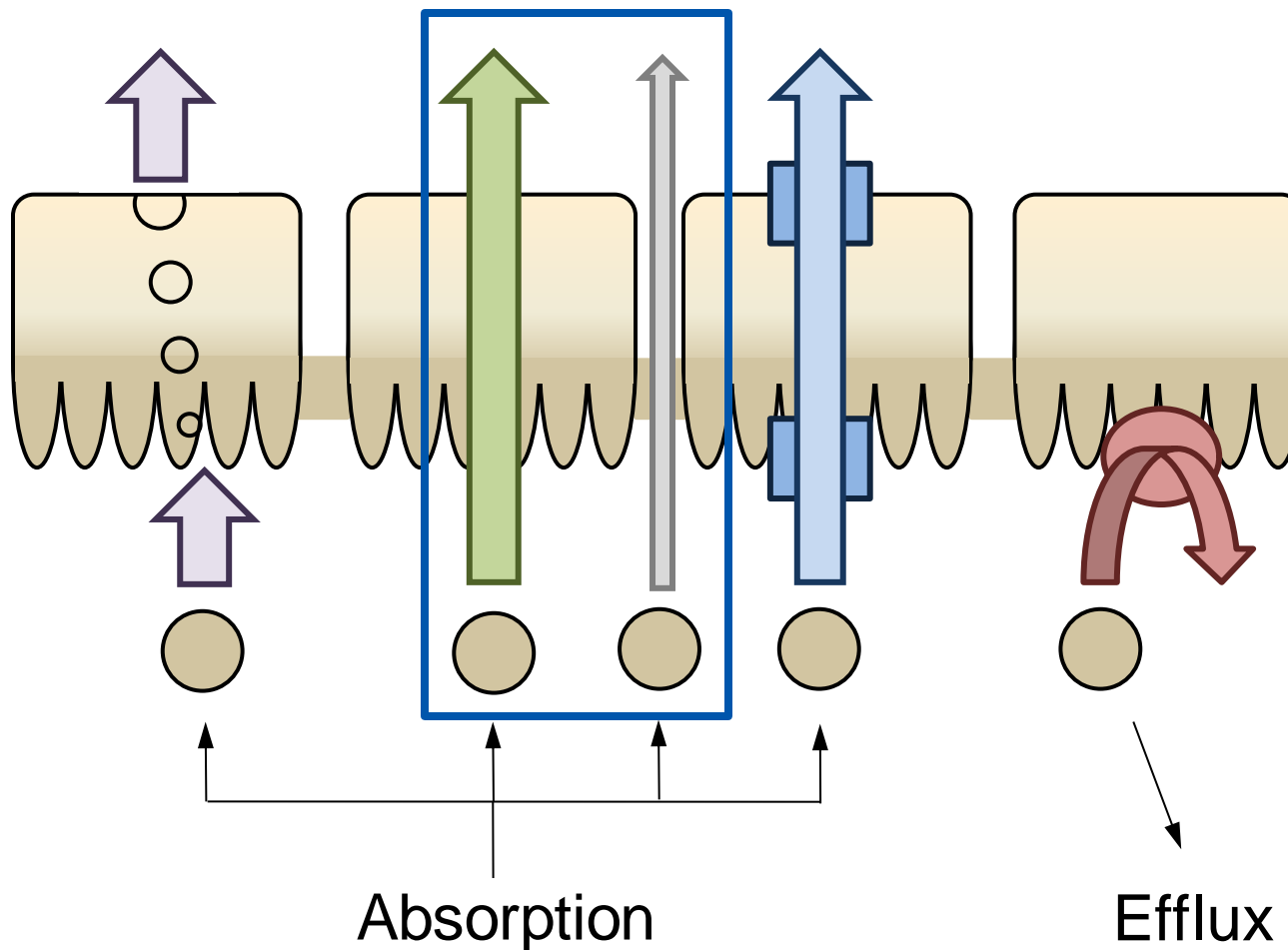
Visionary Software

Advancing Research

# Introduction

- ④ Why are *in silico* predictions needed?
  - ④ Partial replacement of experimental measurements
  - ④ Validation of the results obtained from *in vitro* studies
  - ④ Ability to estimate permeability of a compound prior to synthesis

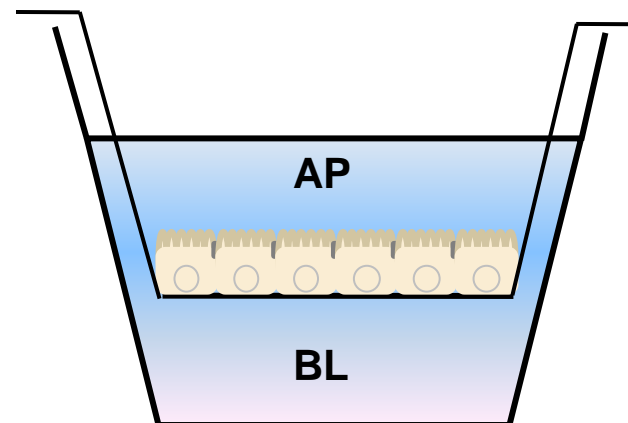
# Monolayer Permeation Mechanisms



# Experimental Data

- 🌀 Caco-2 permeability data set: 682  $P_c$  values for 511 compounds corresponding to passive diffusion
- 🌀 Values affected by carrier-mediated processes excluded from modeling
- 🌀 Data represent  $P_c$  values at a variety of experimental conditions:

Parameter	Range
pH	4 – 8
Stirring rate	0 – 1100 rpm

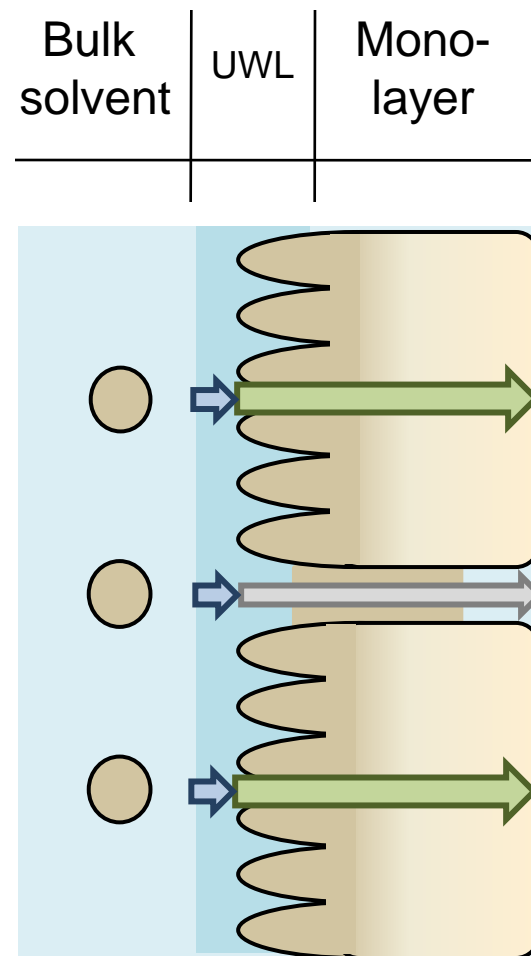


# Theory: Introduction

⦿ A molecule must overcome two barriers to permeate through monolayer

⦿ Overall resistance to diffusion:

$$\frac{1}{P_c} = \frac{1}{P_{UWL}} + \frac{1}{P_{para} + P_{trans}}$$

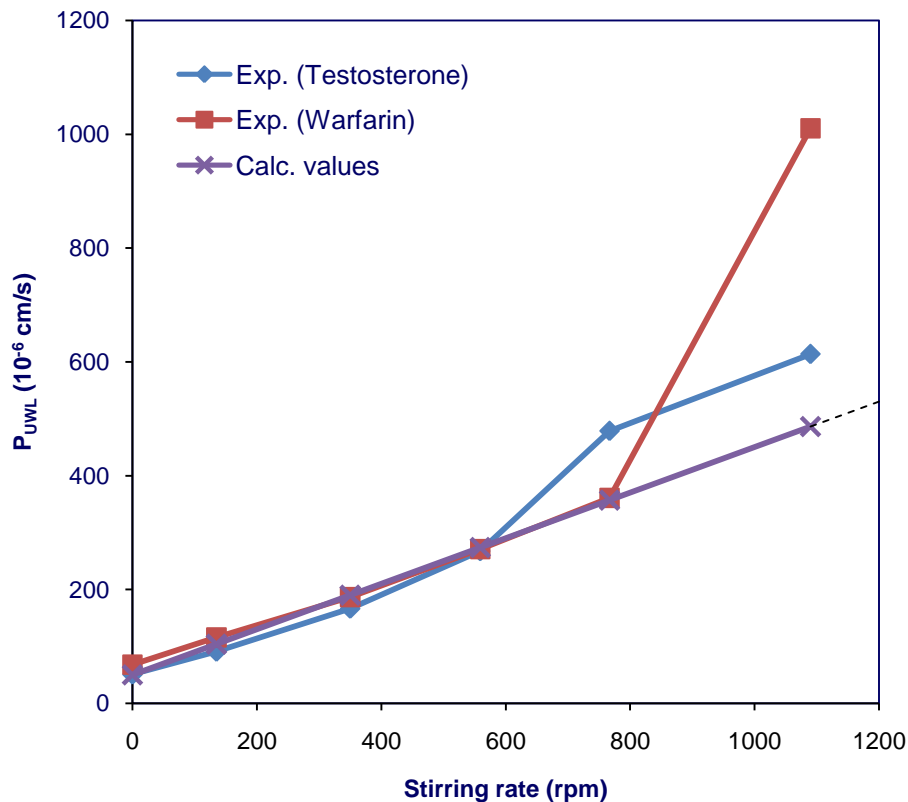


# UWL permeability

•  $P_c$  of testosterone and warfarin measured at different stirring rates

•  $V_s = 0 \div 1090$  rpm

$$P_{UWL} = u \cdot V_s + u_o$$



Exp. values taken from  
Karlsson & Artursson. *Int J Pharm.* **1991**;71:55-64.

# Paracellular permeability

-  Molecular size-restricted diffusion through aqueous pores in tight junctions:

$$P_{para} = \sum_{z=-2}^{+2} C_{para} \cdot F(r/R) \cdot \left( \frac{\kappa(z)}{1 - e^{-\kappa(z)}} \right)$$

Parameter	Caco-2	Human jejunum
Pore size (R)	4.7 Å	5.6 Å
Potential drop	57 mV	80 mV

# Transcellular permeability

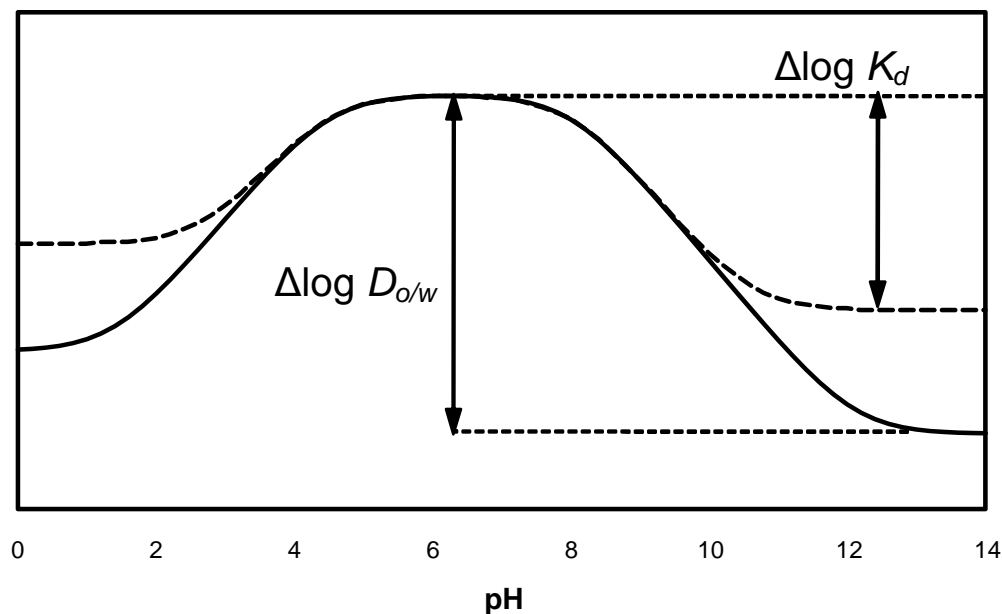
- Considering Fick's law and bilayer/water partitioning:

$$P_{trans} = C_{mem} \cdot D \cdot K_d$$

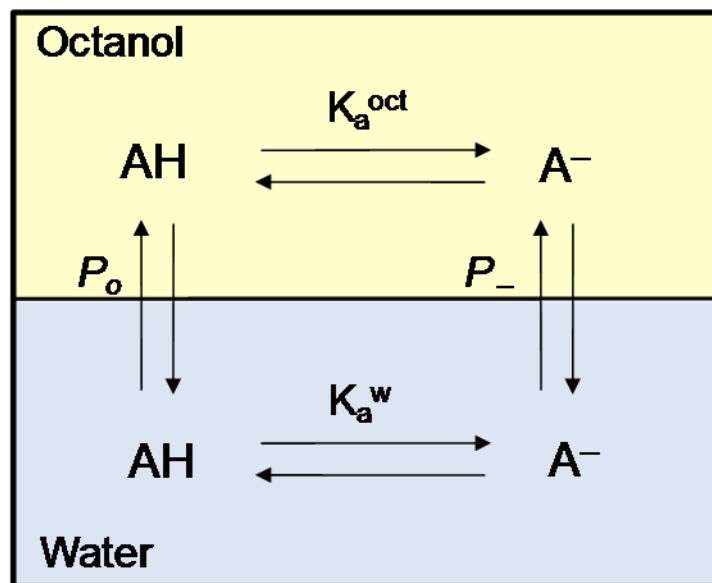
- $C_{mem}$  constant:
  - Bilayer properties
- $D$  coefficient:
  - Molecular size
- $K_d$  constant :
  - Lipophilicity
  - Ionization

# Theory: Ionization (1)

- Accounting for the effect of ionization:
  - Substituting  $\log P_{o/w}$  with  $\log D_{o/w}$
  - Separate estimation of ionization dependence for each considered property

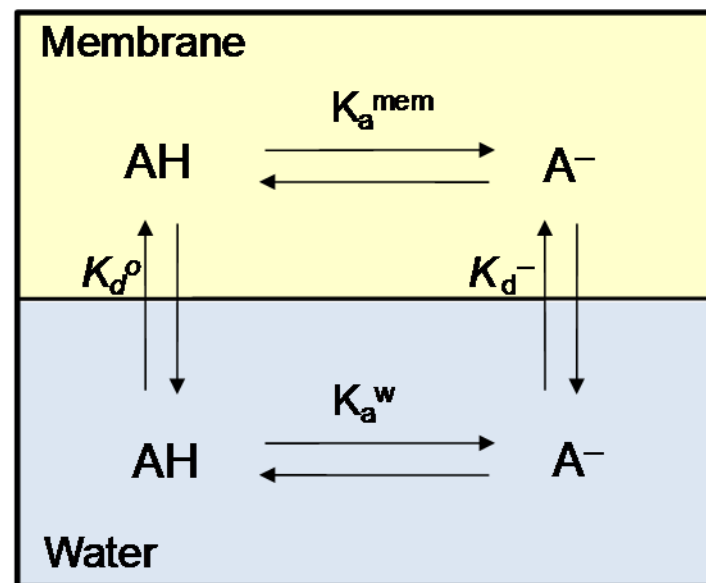


# Theory: Ionization (2)



$$P_o = \frac{[AH]^{oct}}{[AH]^w} \quad P_- = \frac{[A^-]^{oct}}{[A^-]^w}$$

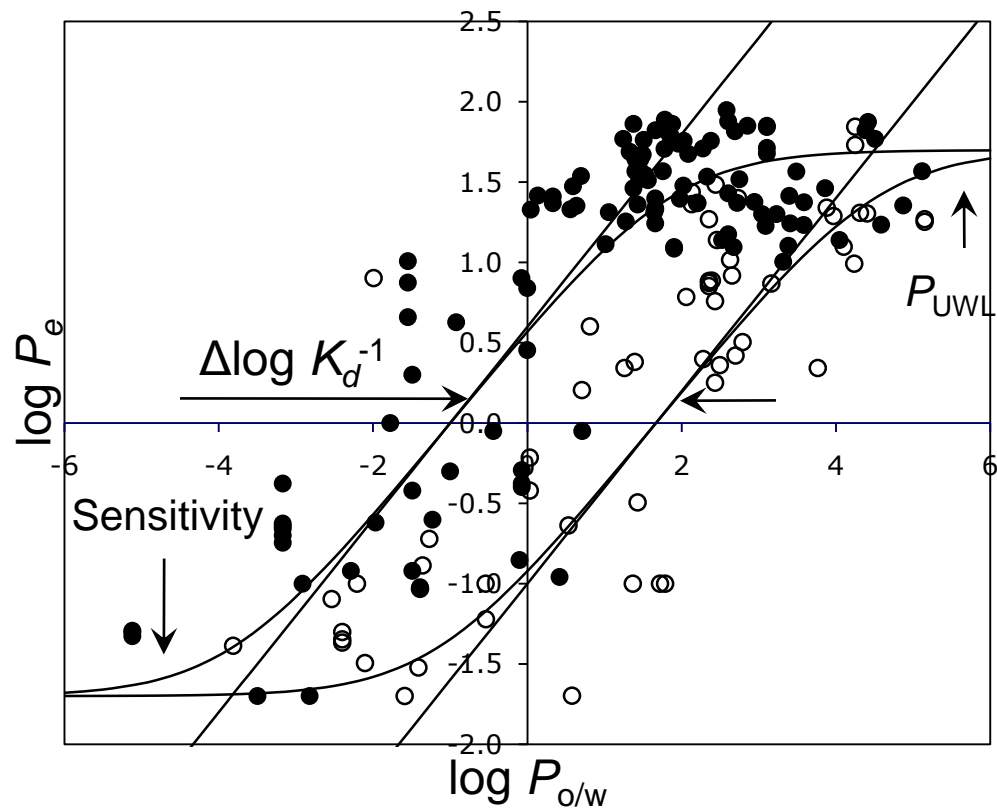
$$\Delta \log D_i = \log P_o - \log P_i$$



$$K_d^o = \frac{[AH]^{mem}}{[AH]^w} \quad K_d^- = \frac{[A^-]^{mem}}{[A^-]^w}$$

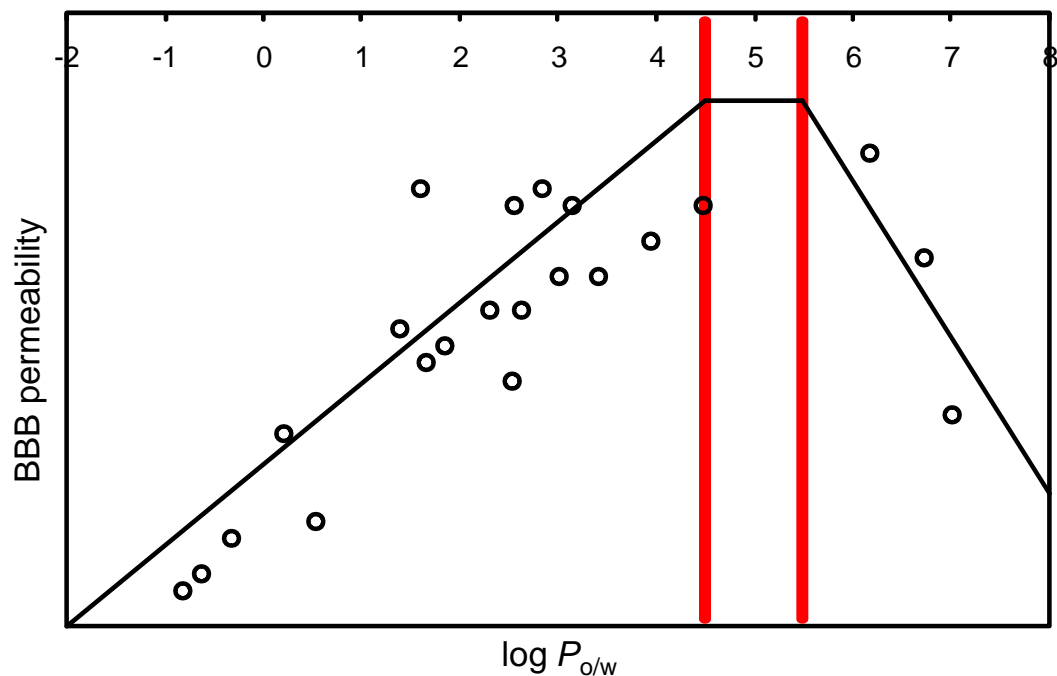
$$\Delta \log K_d^i = \log K_d^o - \log K_d^i$$

# Theory: Lipophilicity



# Theory: Bilinear log $P$

- Very hydrophobic molecules are trapped in the membrane
- Best described by bilinear relationship with  $\log P_{o/w}$



# Model development

## ⊕ Multi-step non-linear fitting

- ⊕ Determination of  $P_{\text{para}}$  parameters
- ⊕ Estimation of  $P_{\text{trans}}$  of non-electrolytes
- ⊕ Evaluation of ionization dependence of  $P_{\text{trans}}$

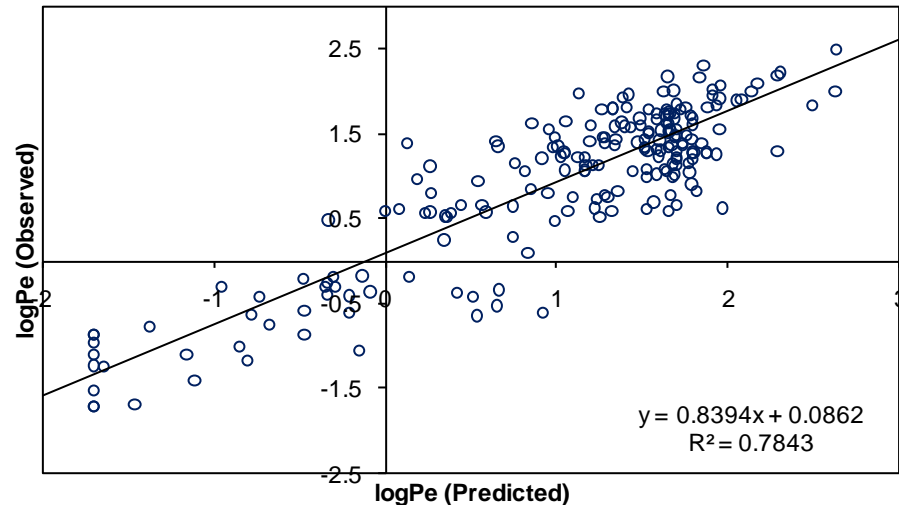
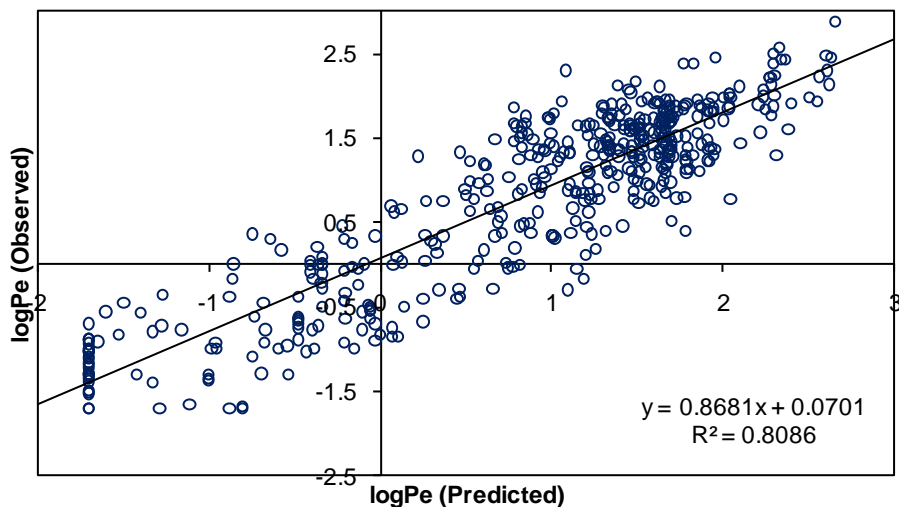
# Results

Parameters		Values			
		OCT	LIP	HIA	CACO-2
Lipophilic increase	$c_1$	+1.0	+0.8	+0.8	+0.6
Hydrogen bonding	$c_2$	—	—	-0.4	-0.4
Lipophilic decrease	$a$	—	—	—	-1.6
Nonlinear LogP term	$\beta$	—	—	—	$10^{-4}$
Ionization (Trans. route)	$\Delta_{\pm}/c_1$	-2.5	—	-2.0	-1.3
	$\Delta_{+}/c_1$	-3.1	-1.25	-2.3	-1.8
	$\Delta_{-}/c_1$	-4.1	-2.5	-3.3	-2.8
	$\Delta_{-2}/c_1$	-5.0	—	—	-4.3

# Model statistics

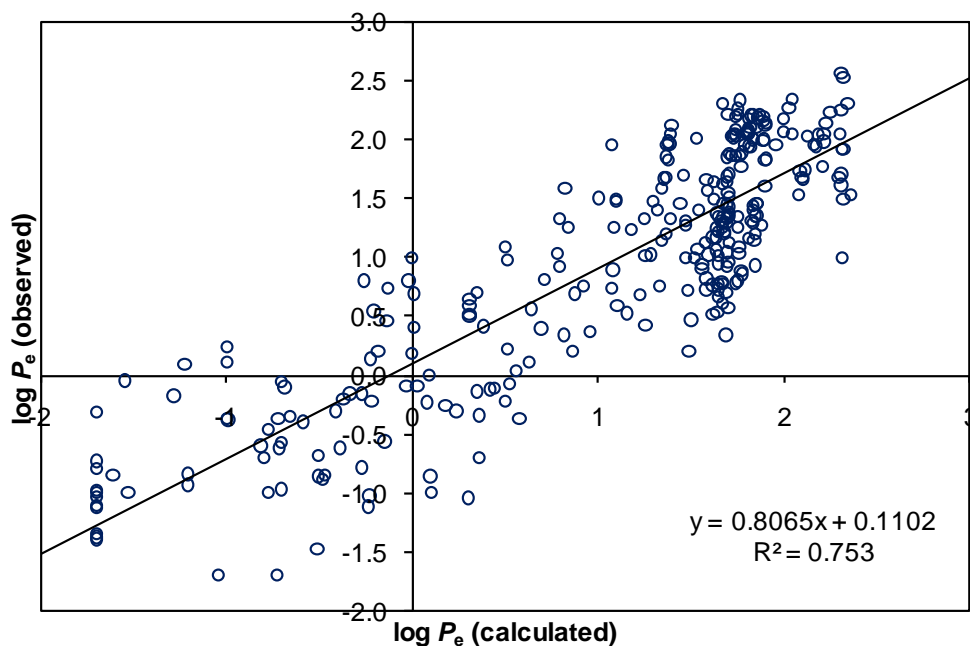
- Training set:
- 473 data points
- $R^2 = 0.80$
- RMSE = 0.50

- Test set:
- 209 data points
- $R^2 = 0.78$
- RMSE = 0.48



# External validation of the model

- 300 additional data points collected when model development was finished
- $R^2 = 0.75$ ;  $RMSE = 0.55$



# Conclusion

- ④ Permeation across Caco-2 monolayers involves overcoming two barriers – UWL and cell layer.
- ④ Permeation by both paracellular and transcellular routes can be reliably modeled using simple physicochemical descriptors – lipophilicity, ionization, and molecular size.
- ④ Ionization dependence of transcellular permeability should be specifically determined for the system under study. Use of  $\log D_{o/w}$  as a descriptor is not appropriate.