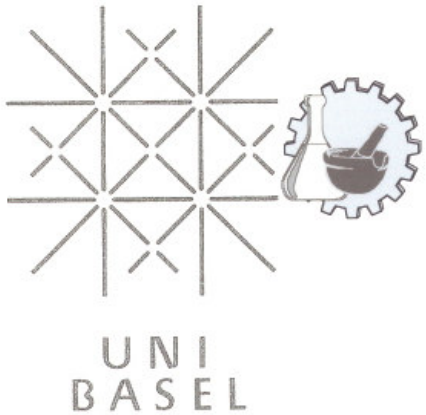

DEVELOPMENT OF A MATHEMATICAL MODEL FOR DETERMINING DRUG ABSORPTION PARAMETERS IN CACO-2 CELL MONOLAYERS

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Purpose

- Develop and evaluate a mathematical model for delineating the passive and carrier mediated contributions to the absorption of some apical efflux substrates in Caco-2 cell monolayers.

- Evaluate the effect of concentration and binary combinations of substances, on permeation in terms of these two contributing factors, passive diffusion and active apical efflux.

- Integrate phase II metabolism and adsorption to the Transwell® surface to the model.

- The objective was to overcome the limitations of the usually employed simple analysis that is based on calculation of the P_{app} in apical to basal and basal to apical directions and the efflux ratio.

Methods

- Caco-2 cell monolayers grown in 6-well Transwells® for 18 to 21 days were used.
- P-glycoprotein substrates Digoxin, Quinidine, Verapamil and Amentoflavone were used alone or in binary combinations in different concentrations.
- Bidirectional transport was studied and the used compounds were quantified by HPLC.
- The system of differential equations of the mathematical model was fitted to experimental concentration data, and optimal values of the kinetic parameters were deduced using the software EASY-FIT (Prof. K. Schittkowski, University of Bayreuth, Germany).

Conventional P_{app} approach and Efflux Ratio

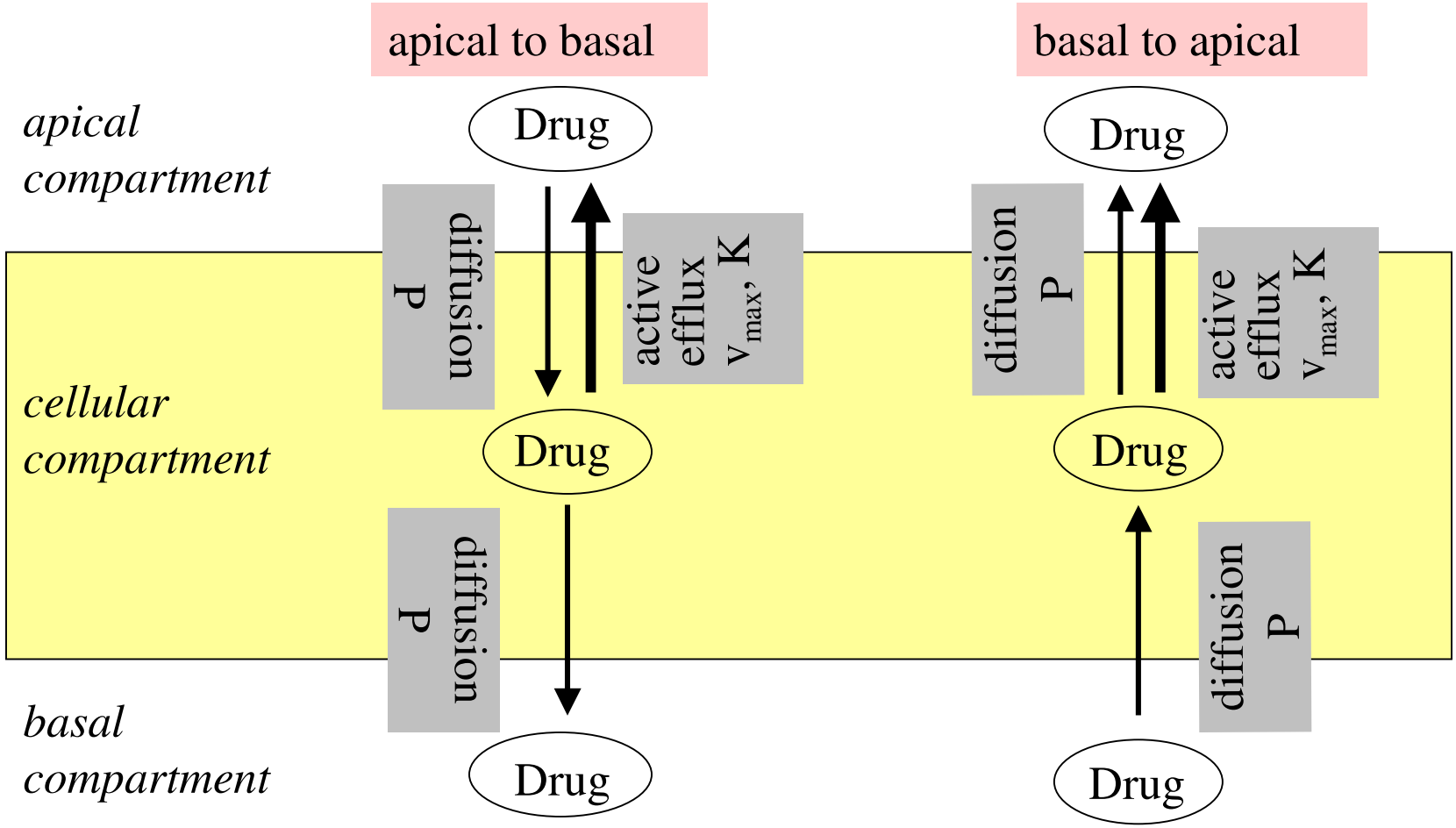
$$P_{app} = \frac{V_R * \Delta C_R}{\Delta t * A * C_{D0}}$$

- Initial drug amount in the donor
- Linear drug transport with time applicable to early time points only
- Assumes negligible back flux and no mass balance problems

$$EffluxRatio = \frac{P_{app:basal\_to\_apical}}{P_{app:apical\_to\_basal}}$$

- Time dependent
- Some substrates with high passive permeability (e.g. Verapamil) may not be detected by this approach
- No separate quantification of the passive permeability and the apical efflux
- No direct quantification of the influence of inhibitors and/or concurrently applied compounds

Theoretical modeling: Transport processes considered for modeling drug permeation in the Caco-2 monolayer and relevant processes



Theoretical modeling: assumptions

- The change of concentration in the apical, the cellular and the basal compartment is considered

- Drug movement between the compartments by passive diffusion through the apical and the basal cell membrane is symmetrical and is characterized by the permeability coefficient, P

- Drug is subject to carrier mediated active efflux from the cellular to the apical compartment. This follows saturable kinetics which may be characterized by the kinetic parameters, v_{max}, and K,

- The drug concentration in the apical compartment does not influence efflux transport and the entire mass of drug present in the cellular compartment is substrate of the transporter

- Mass is preserved

differential equations describing the change of drug concentration or mass as a function of time in the three compartments during permeation in both directions

transport direction apical to basal

$$\frac{dC_{Aab}}{dt} = -P * (C_A$$

$$\frac{dC_{Bab}}{dt} = P * (C_{Ca}$$

$$\frac{dM_{Cab}}{dt} = P * (C_A$$

$$\frac{v_{\max} * M_{Cab}}{K + M_{Cab}} \approx vk$$

transport

$$\frac{dC_{Aba}}{dt} = P * (C_{Cb}$$

$$\frac{dC_{Bba}}{dt} = -P * (C_B$$

$$\frac{dM_{Cba}}{dt} = P * (C_{Bba} - C_{Cba}) * Sm - P * (C_{Cba} - C_{Aba}) * Sm - \frac{v_{\max} * M_{Cba}}{K + M_{Cba}} * Sm$$

compartment

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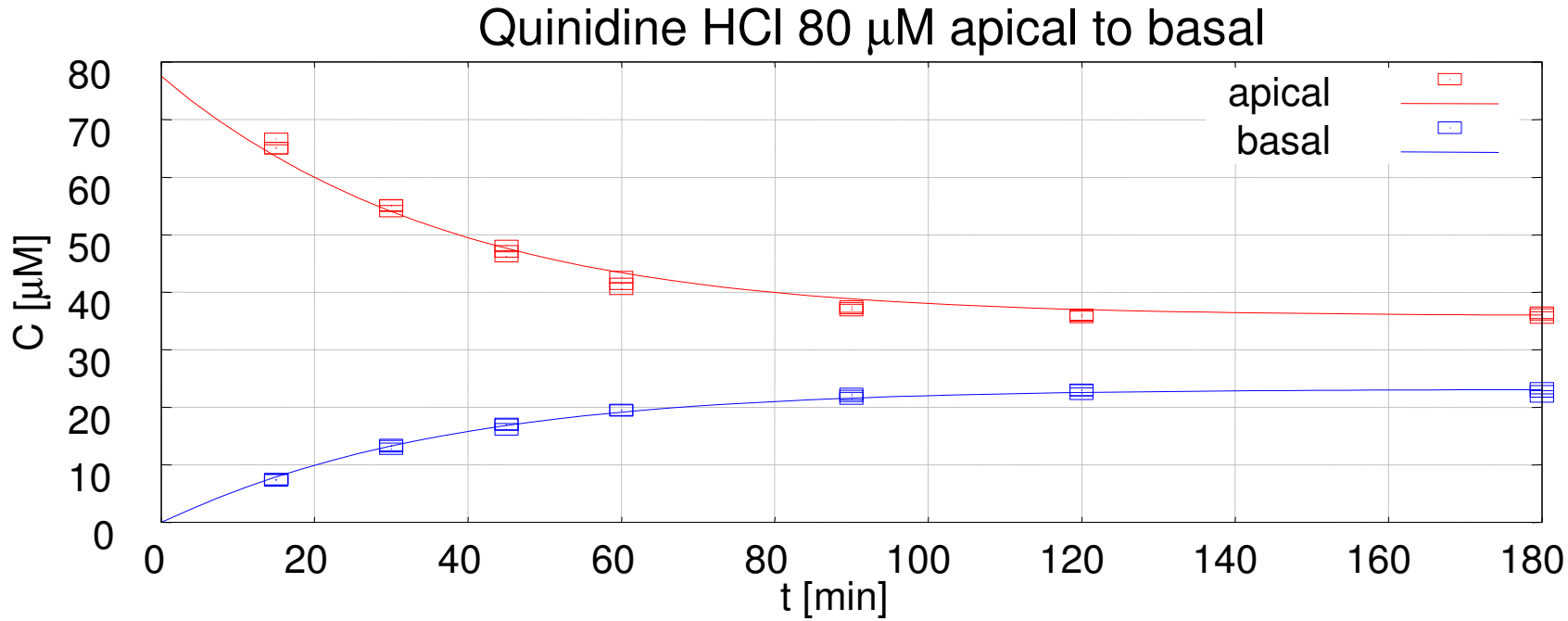
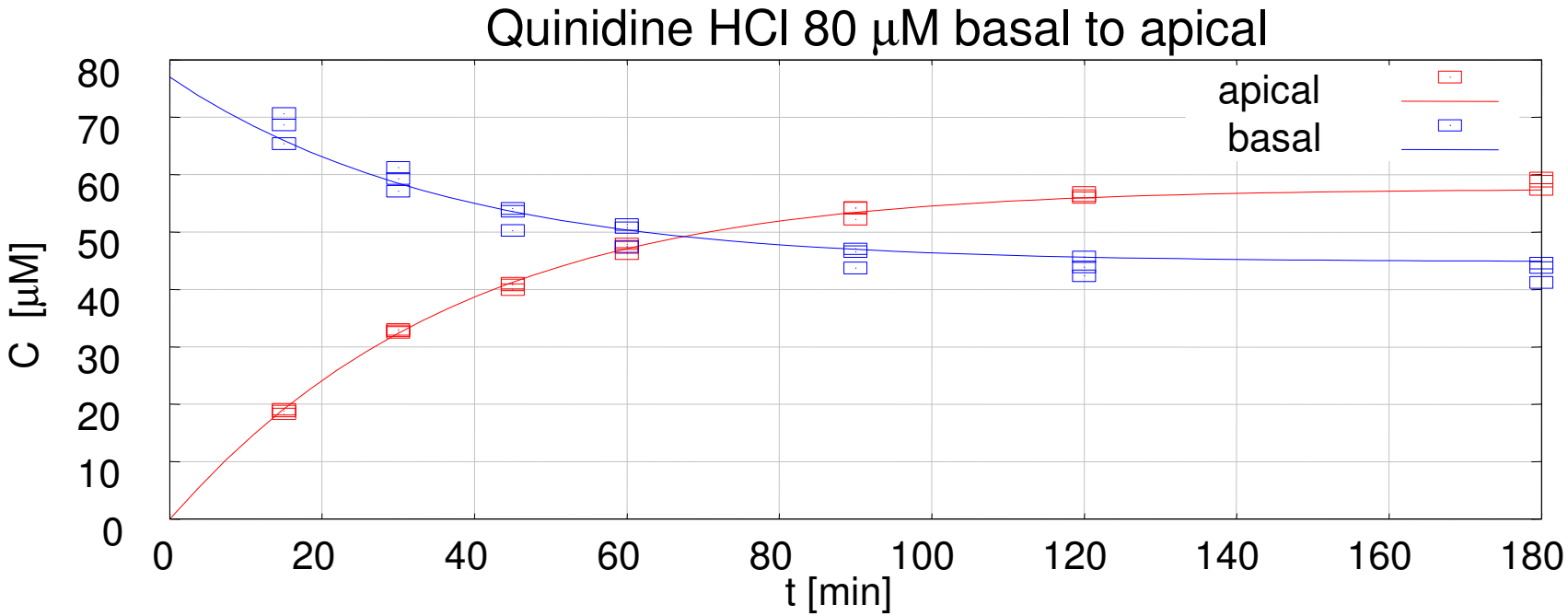
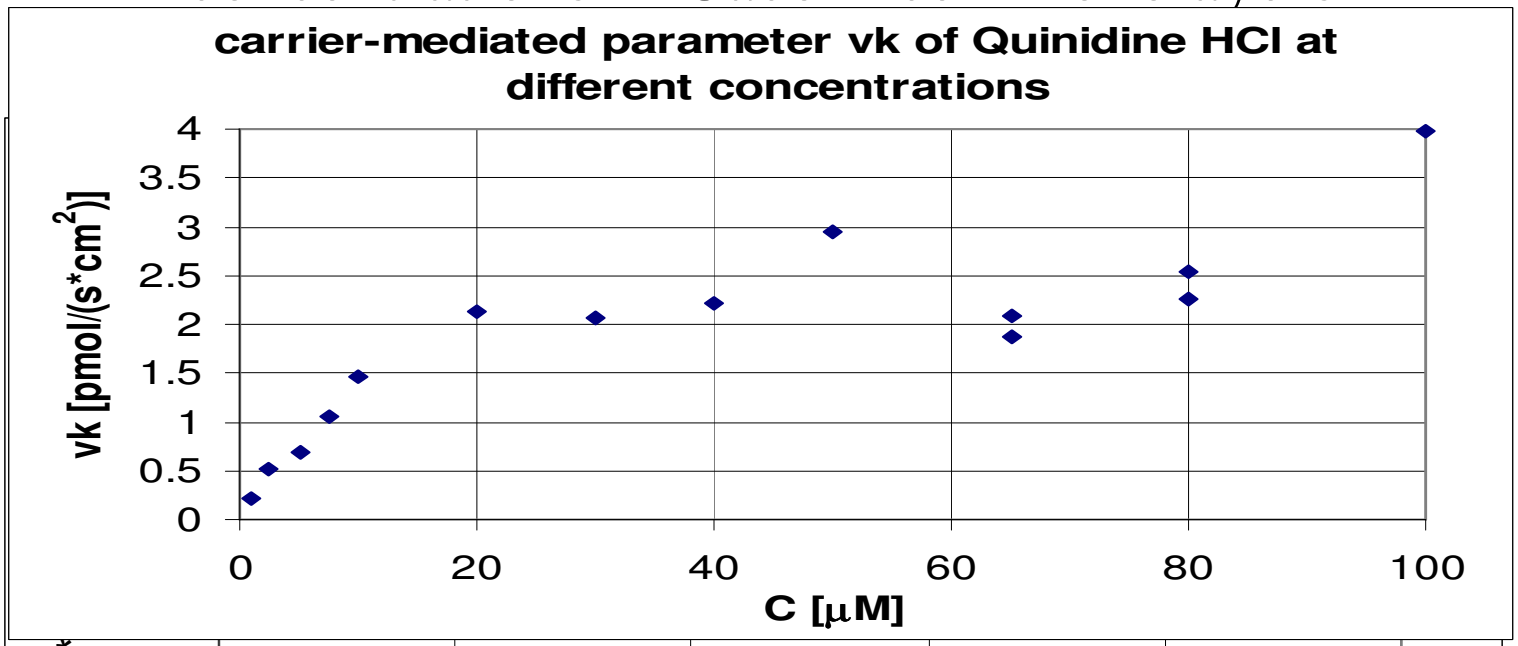
cellular

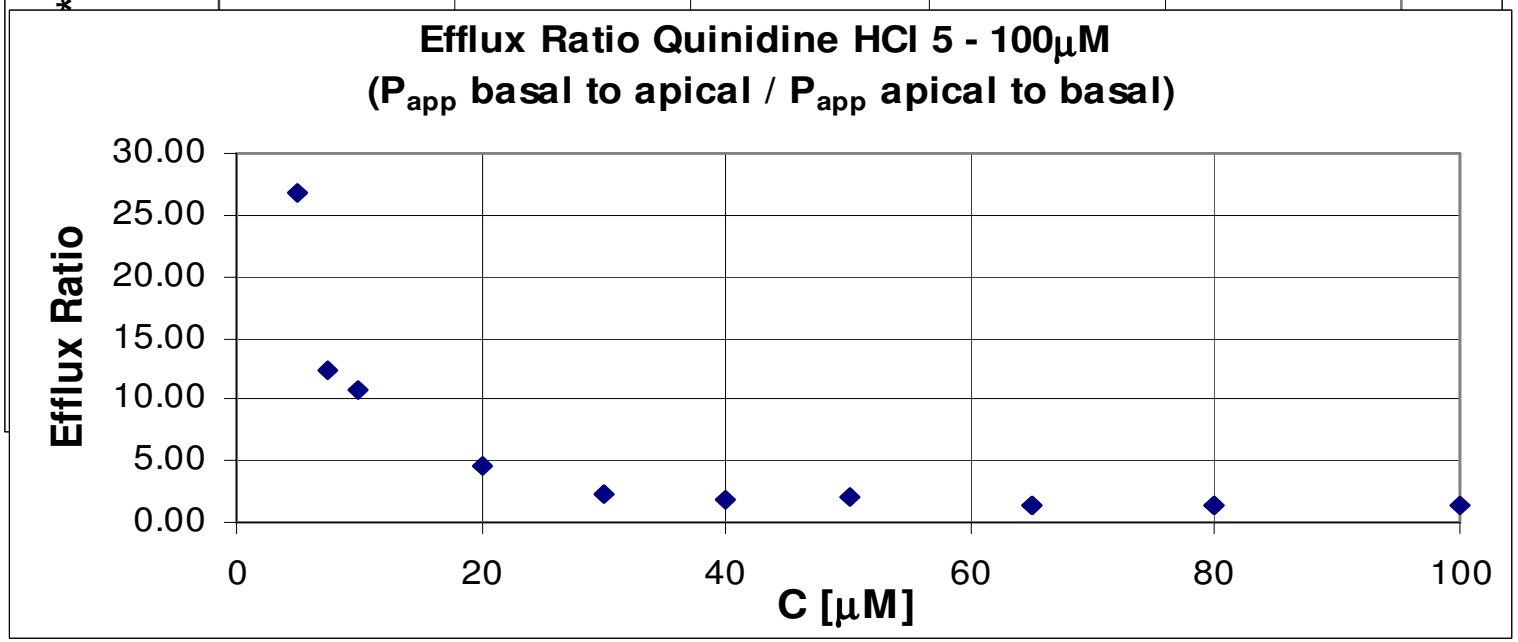
representative fits of Quinidine HCl in both transport directions





Passive and carrier-mediated transport of different Quinidine HCl concentrations in Caco-2 cell monolayers

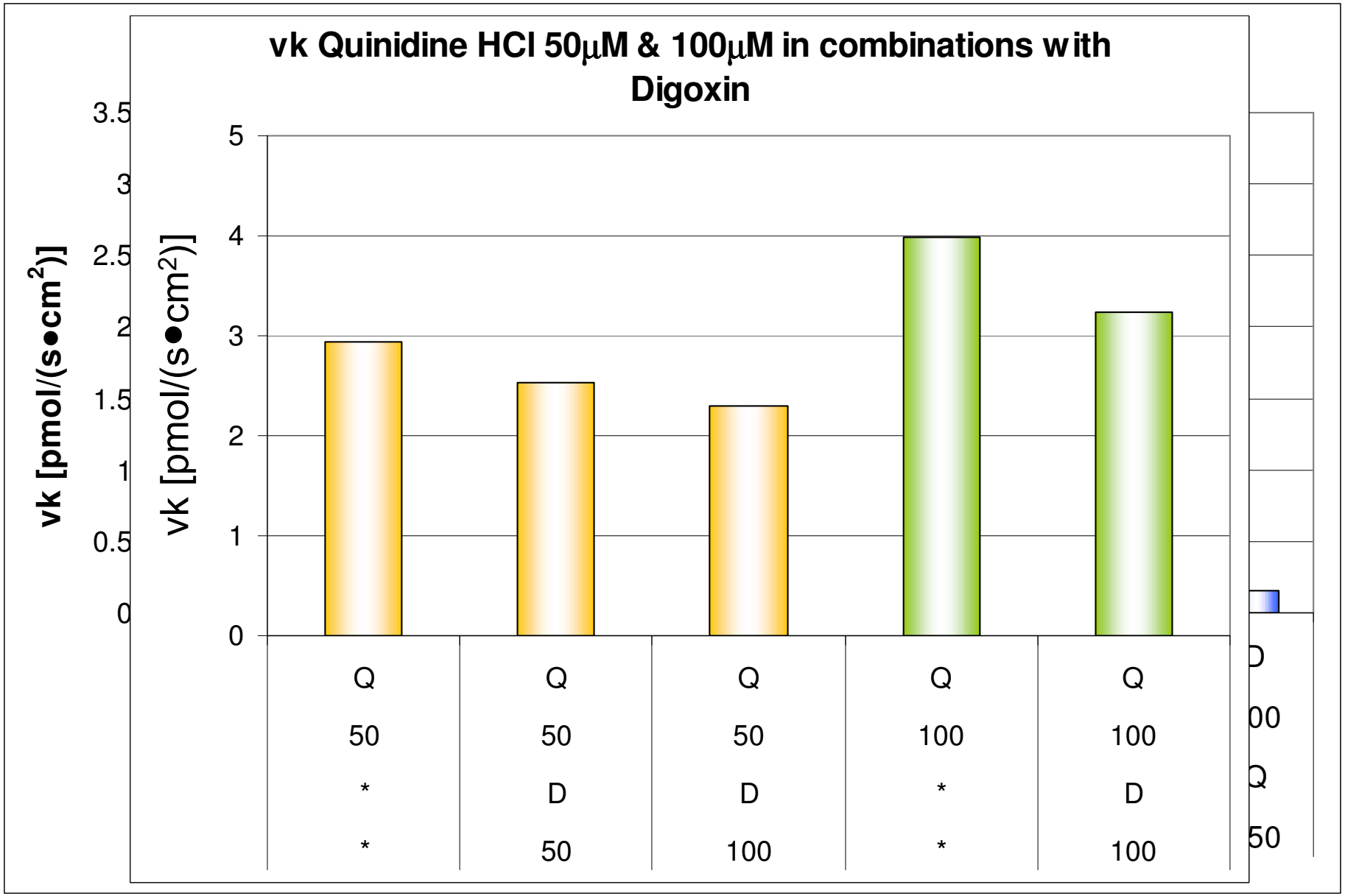




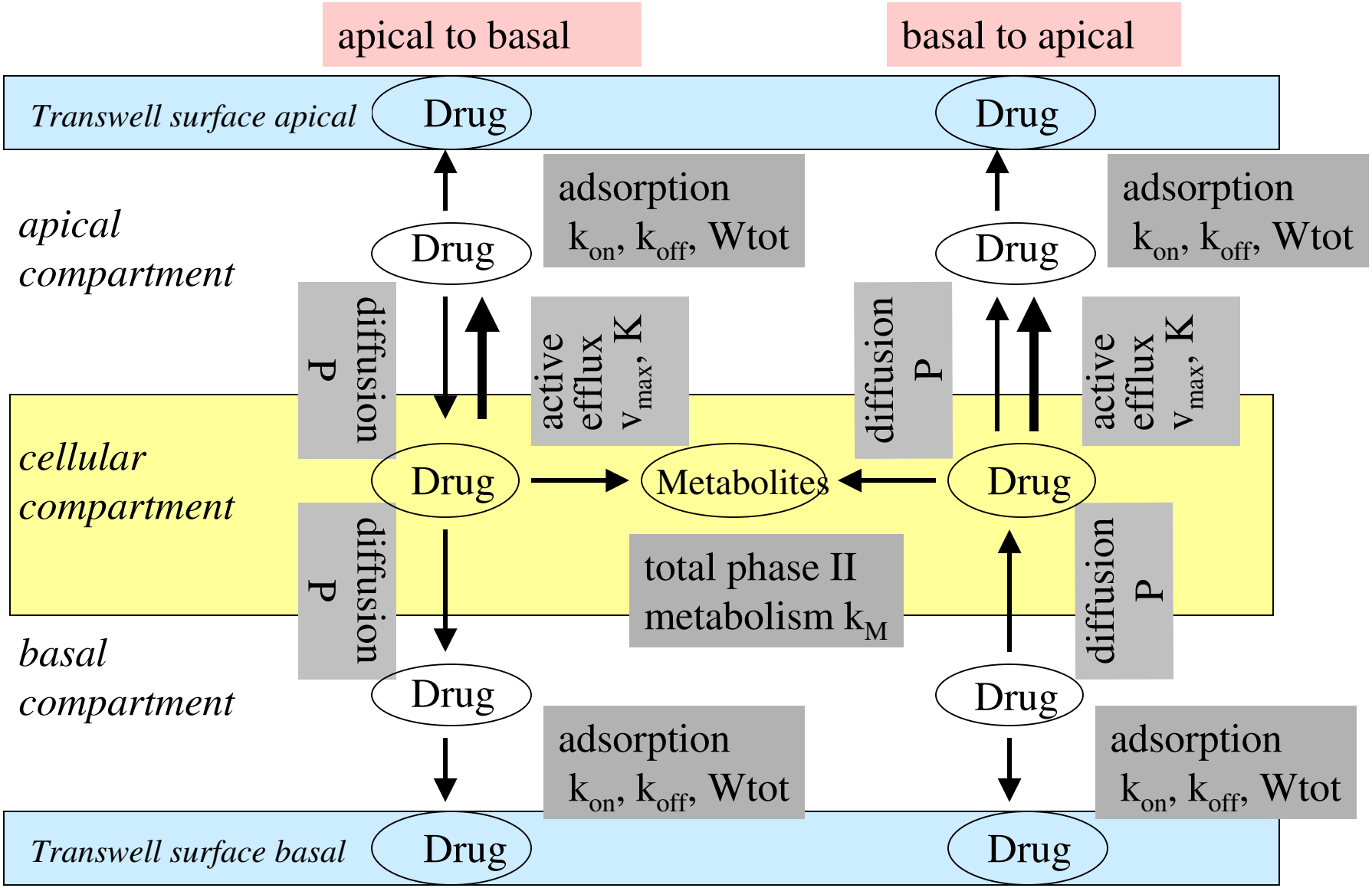
Kinetic parameters of transport I

	Quinidine HCl	**Digoxin**	**Verapamil HCl**
C_{D0} [µM]	50	50	50
P • 10^6 [cm/s]	206.74	25.97	293.29
vk [pmol/(s•cm²)]	2.941	1.438	1.281
average Mcell a to b [nmol]	0.137	0.004125	0.144
average Mcell b to a [nmol]	0.210	0.043	0.225

Kinetic parameters of transport II – transport inhibition



Transport processes considered for modeling drug permeation in the Caco-2 monolayer and relevant processes including phase II metabolism and adsorption to Transwell surface



differential equations describing the Amentoflavone transport including adsorption and phase II metabolism as a function of time

transport direction apical to basal

compartment

$$\frac{dC_{Aab}}{dt} = -P*(C_{Aab} - C_{Cab})*Sm/V_A + vk*M_{Cab}*Sm/V_A - k_{on}*C_{Aab}*(W_{tot} - C_{WAab}) + k_{off}*C_{WAab}*S_A/V_A$$ apical

$$\frac{dC_{Bab}}{dt} = P*(C_{Cab} - C_{Bab})*Sm/V_B - k_{on}*C_{Bab}*(W_{tot} - C_{WBab}) + k_{off}*C_{WBab}*S_B/V_B$$ basal

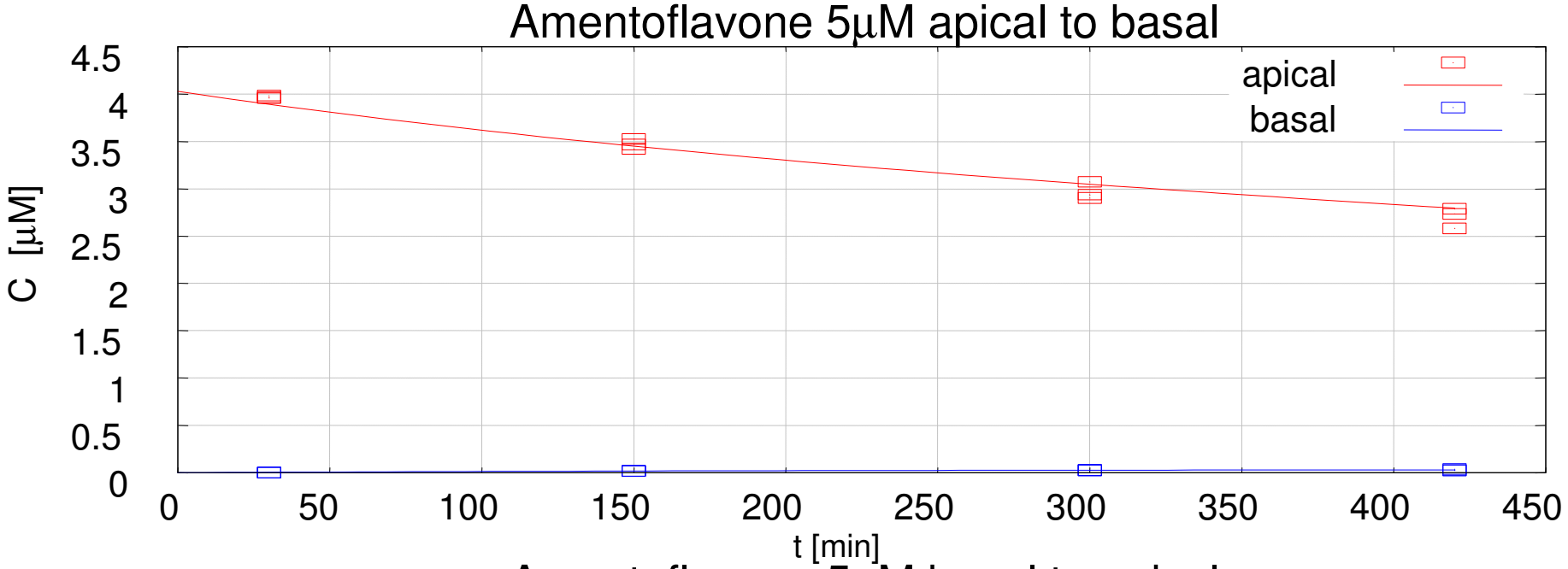
$$\frac{dM_{Cab}}{dt} = P*(C_{Aab} - C_{Cab})*Sm - vk*M_{Cab}*Sm - P*(C_{Cab} - C_{Bab})*Sm - k_M*M_{Cab}$$ cellular

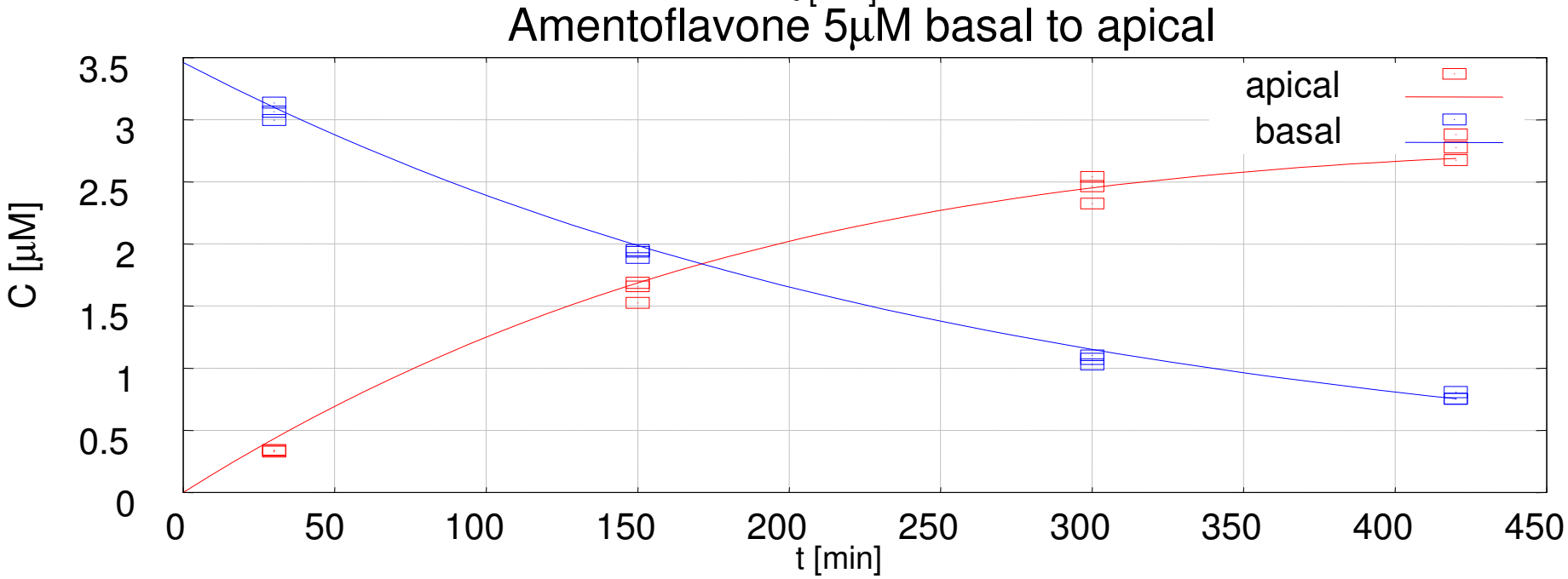
$$\frac{dM_{MCab}}{dt} = k_M*M_{Cab}$$ phase II metabolism

$$\frac{C_{WAab}}{dt} = k_{on}*C_{Aab}*(W_{tot} - C_{WAab})*V_A/S_A - k_{off}*C_{WAab}$$ Transwell apical

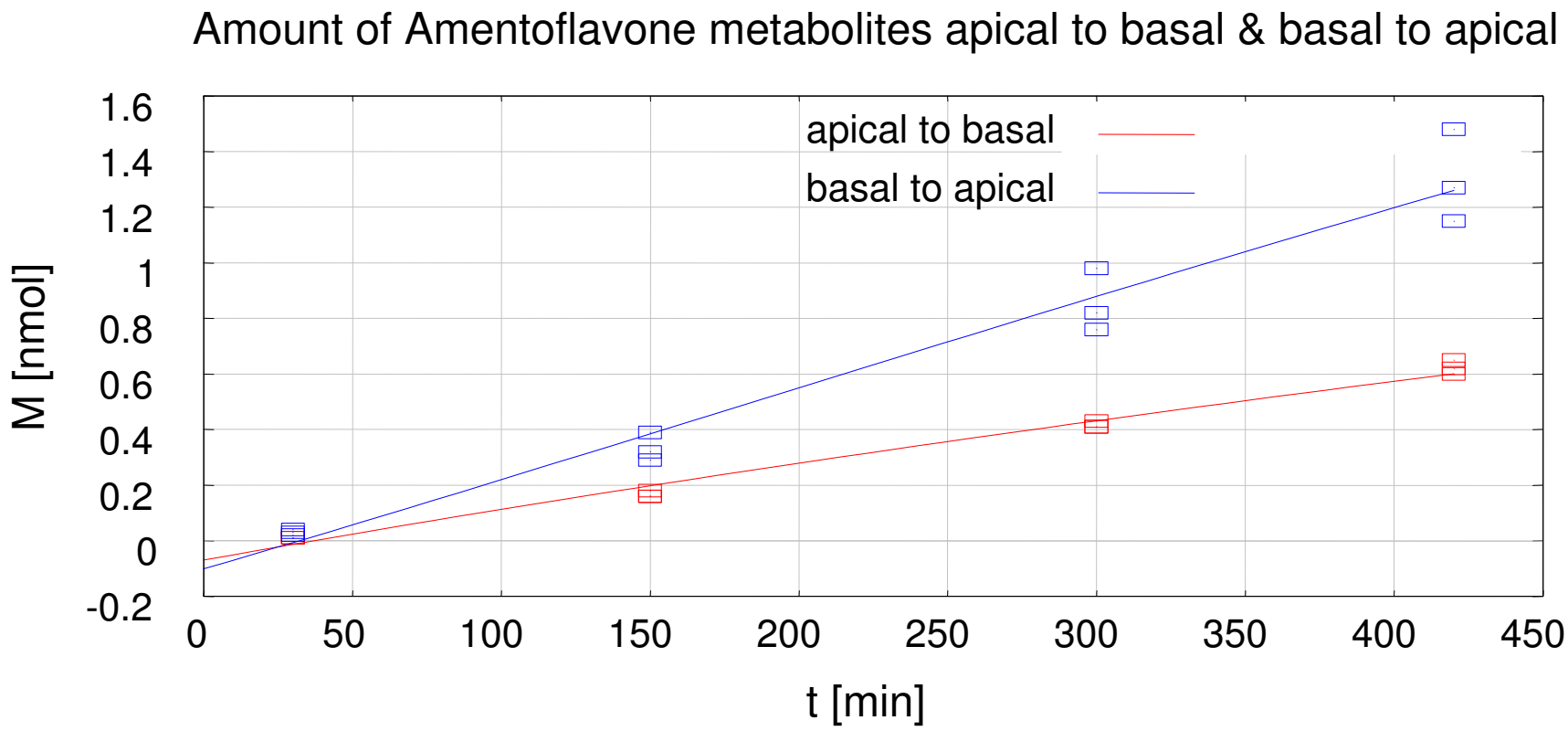
$$\frac{C_{WBab}}{dt} = k_{on}*C_{Bab}*(W_{tot} - C_{WBab})*V_B/S_B - k_{off}*C_{WBab}$$ Transwell basal

fits of Amentoflavone transport in both directions





fits of Amentoflavone metabolites for transport in both directions



Conclusions

- All compounds used individually showed a polarised transport behaviour, the extent of which depended on the drug and the concentration used
- Kinetic parameters of the transport could be calculated with the developed model which included passive and carrier mediated transport, metabolism and non-specific adsorption
- Model estimated passive permeability coefficient of each drug was independent of concentration and varied between the drugs
- Model estimated apical efflux parameters varied between the different drugs and expressed the mass efflux rate of these drugs elicited by P-glycorprotein in Caco-2 cells
- The apical efflux parameter also reflected inhibition of the efflux by concomitant drugs
- Phase II metabolic rate constant in the cell was estimated
- By integrating surface adsorption, cellular transport was corrected for this distorting effect