# *In silico* modelling of *in vitro* bidirectional transport studies and comparison to conventional data analysis

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## Introduction

Estimates of effective drug permeability that are used in PBPK models are often derived from apparent permeability ($P_{app}$) estimates from *in vitro* Transwell® studies. The conventional analysis of such studies assumes that sink conditions are maintained, which can be difficult to achieve experimentally, particularly for high permeability compounds. Modelling approaches which account for the changes in drug concentration in both donor and receiver wells over time are not limited by this assumption [1,2]. However, the number of studies which provide a side-by-side comparison of these data analysis techniques is limited. In the current study, bidirectional transport experiments for metoprolol, a high permeability compound with no active transport, were analysed using the conventional approach and with a three-compartment (3C) model.

## Aims

- To simultaneously fit multiple time-point data for metoprolol bidirectional transport across Caco-2 monolayers in both apical-to-basolateral (A-B) and basolateral-to-apical (B-A) transport directions.
- To investigate the impact of dynamically accounting for the changes to receiver well concentrations upon buffer sampling within a 3C model.
- To investigate the ability of a 3C model to account for the impact of adding bovine serum albumin (BSA) at concentrations ranging from 1 to 4% into the basolateral well.

## Methods

*Bidirectional Transport Studies:*

Data for the bidirectional transport of metoprolol across Caco-2 monolayers was previously generated [3]. Briefly, Caco-2 cells were seeded at a density of 1 x $10^5$ cells/well onto Transwell inserts and grown for 20 days prior to transport experiments. Transport experiments were performed at 37°C using HBSS with 10 mM Hepes (pH 7.4) buffer at volumes of 0.5 and 1.5 mL in apical and basolateral compartments, respectively. In addition, matching experiments were performed in the presence of concentrations of BSA from 1 to 4% w/v in the basolateral buffer (pH 7.4).

Experiments (n = 6 filters) were initiated by adding [$^3$H]-metoprolol to donor buffer at a concentration of 1.1 µM or 1 mM. At 5, 15, 25, 50, 80 and 120 mins, receiver buffer was sampled and replaced with an equal volume of blank buffer.

Sampling of A-B experiments was conducted by moving the Transwell insert to a new well containing blank buffer and retaining the previous well, thereby representing complete removal of drug from basolateral buffer. Sampling of B-A experiments was conducted by removal of 400µl of apical buffer and replacement with an equal volume of blank buffer. [$^3$H]-metoprolol concentrations in the sampled buffer were determined by scintillation counting.

*Data analysis (conventional):*

Apparent permeability ($P_{app}$) for A-B and B-A experiments was determined using conventional methods [3]. Only data in the linear range (up to 25 mins) were used for this analysis and receiver well concentrations were corrected for sampling in a 'static' manner by accounting for the amount of drug removed in the previous sample in the calculation of the receiver well concentration in the following sample.

*Data analysis (modelling):*

Simultaneous fitting of metoprolol receiver well concentrations at all time points of both A-B and B-A experiments was performed. Three scenarios were investigated as outlined in Table 1. In scenario 1, sampling correction was via the 'static' process described above for the conventional analysis, whereas for Scenarios 2 and 3 the model was fitted to uncorrected data but the amount removed from the receiver well upon sampling was accounted for dynamically within the model.

**Table 1.** Details of different scenarios for fitting transport data

| Scenario | Basolateral [BSA] (w/v %) | Sampling Correction | Parameters fitted | Software |
|---|---|---|---|---|
| 1 | 0 | Static | $P_{mem}$ | SIVA |
| 2 | 0 | Dynamic | $P_{mem}$ | R |
| 3 | 0, 1, 2, 4% | Dynamic | $P_{mem}$, $fu_{bl}$, $fu_{ap}$ | R |

In all cases a three-compartment (3C) model was used (Figure 1) in which metoprolol can pass between apical, cell and basolateral compartments via transcellular passive permeability, which is equal for both membranes ($P_{mem}$). With the exception of $fu_{ap}$ and $fu_{bl}$ in Scenario 3, the fraction unbound in apical, cell and basolateral compartments was fixed at 1. Surface area was fixed at the filter area of 1.13 $cm^2$. Cell volume ($V_{cell}$) was calculated from cell protein content and a scaling factor of 3.65 µL/mg protein [4]. Nonlinear weighted least squares (WLS) estimation ($1/Y_{pred}^2$) was performed using either the Simcyp *in vitro* analysis toolkit (SIVA version 1.0, Simcyp, Sheffield, UK) or R (version 3.1.0, R Foundation for Statistical Computing).

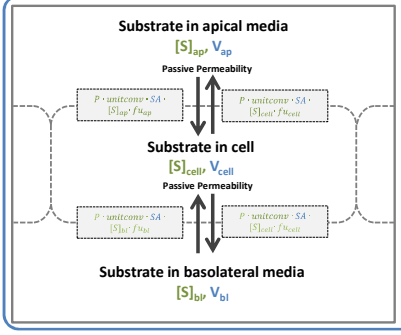


**Figure 1.** 3C model used to fit metoprolol transport data. System-specific parameters are coloured blue, drug-specific parameters are coloured green.

Where:
[S] = Substrate (metoprolol) concentration (µM)
P = Passive permeability ($10^{-6}$ cm/sec)
fu = Fraction unbound
SA = Surface area ($cm^2$)
V = Volume (µL)
unitconv = Unit conversion for P (60/1000)
Subscripts ap, bl and cell, refer to apical, basolateral and cell values, respectively

## Results

*Conventional analysis:*

In the absence of added BSA, at a donor metoprolol concentration of 1.1 µM the mean (95% confidence interval) $P_{app}$ values obtained via conventional data analysis were 107 (104 – 110) and 99 (95 – 104) x $10^{-6}$ cm/s for A-B and B-A directions, respectively. Corresponding $P_{app}$ values with a donor metoprolol concentration of 1000 µM and at different basolateral BSA concentrations are shown in Figure 2.

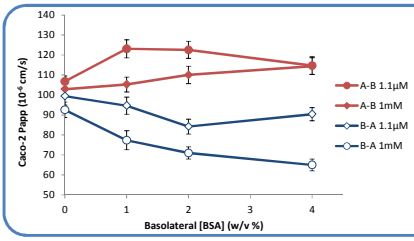


**Figure 2.** Metoprolol $P_{app}$ values for A-B and B-A transport determined at different basolateral BSA concentrations using conventional analysis.

*3C Model (Scenario1):*

When the 3C model was fitted to A-B and B-A metoprolol receiver concentrations after 'static' correction for sampling, time points up to 25 mins were well recovered. However, later time points, when the sampling gaps were >10 mins, were fitted poorly (Figure 3). The resulting $P_{mem}$ estimates are shown in Table 2.

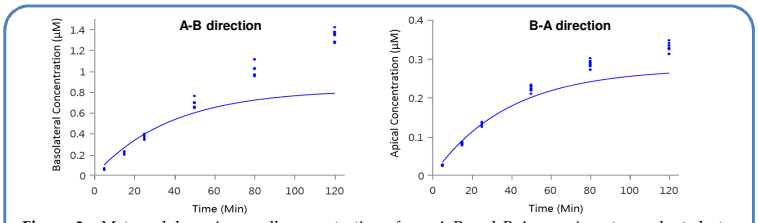


**Figure 3.** Metoprolol receiver well concentrations from A-B and B-A experiments conducted at a donor concentration of 1.1µM (points) and results of fitting the 3C model in SIVA (line). Data were corrected for the effect of sampling but this was not dynamically incorporated into the model.

*3C Model (Scenario2):*

When the 3C model was fitted to A-B and B-A metoprolol receiver concentrations with 'dynamic' correction for sampling, an improved fit to all time points was achieved (Figure 4). The resulting $P_{mem}$ estimates are shown in Table 2.

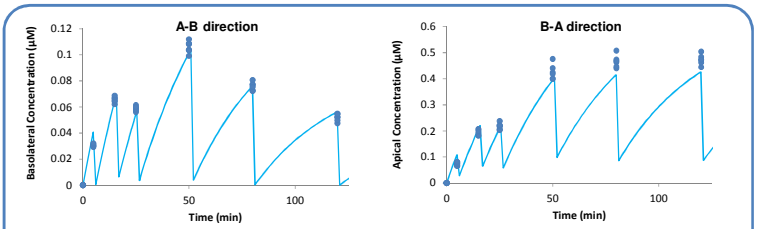


**Figure 4.** Metoprolol receiver well concentrations from A-B and B-A experiments conducted at a donor concentration of 1.1µM (points) and results of fitting the 3C model in R (line). Data were not corrected for sampling, however the effect of sampling was incorporated dynamically into the model.

| Scenario | Donor [Metoprolol] (µM) | Caco-2 $P_{mem}$ $10^{-6}$ cm/s (95% CI) |
|---|---|---|
| 1 | 1.1 | 288 (253 – 325) |
| | 1000 | 275 (246 – 303) |
| 2 | 1.1 | 290 (271 – 310) |
| | 1000 | 260 (245 – 275) |

**Table 2.** $P_{mem}$ estimates fitted using a 3C model in the scenarios described in Table 1. Note: The estimated $P_{A-B}$ equals $P_{mem}/2$, which is then comparable to $P_{app, A-B}$.

*3C Model (Scenario3):*

When the 3C model was fitted to A-B and B-A experiments conducted with varying concentrations of BSA added to the receiver well, a decrease in $fu_{bl}$ was identified (Figure 5), whilst $P_{mem}$ and $fu_{ap}$ estimates remained static (< 10% change).

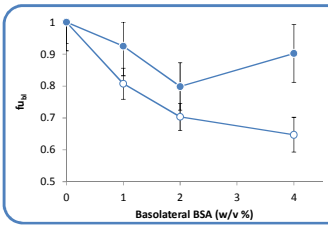


**Figure 5.** Metoprolol $fu_{bl}$ estimates from A-B and B-A experiments conducted at a donor concentrations of 1.1 µM (filled circles) and 1000 µM (open circles) with varying concentrations of BSA in the basolateral well.

With the final 3C model, sensitivity analysis around $V_{cell}$ indicated that a value 10-fold higher or lower than the calculated value had a negligible impact on model outcome.

## Conclusions

This study highlights the impact of dynamically accounting for sampling and replacement in models of *in vitro* permeability studies as this aspect of the experimental design has a significant impact on the maintenance of sink conditions.

## References

1. Heikkinen *et al.*, 2010. *Eur J Pharm Sci* 40: 132-142
2. Korzekwa *et al.*, 2012. *Drug Metab Dispos* 40: 865-876
3. Neuhoff *et al.*, 2006. *Pharm Res* 23(2):350-9
4. Burnham DB and Fondacaro JD (1989) *Am J Physiol* **4** G808-16.