Dissolution-Permeability Apparatus with Integrated *In Situ* Concentration Monitoring of both Donor and Receiver Compartments

K. Tsinman, O. Tsinman; Pion Inc., 10 Cook Street, Billerica, MA 01821, USA

ktsinman@pion-inc.com

INTRODUCTION

Development strategies for insoluble compounds require not only measurement of solubility enhancements achieved through formulation but also assessment of the effect a formulation has on permeability[1]. An new dissolution-permeability measurement platform (µFLUX™) allows simultaneous monitoring of both effects thereby providing an *in vitro* path to early *in vivo* predictive biorelevant absorption testing.

MATERIALS AND METHODS

Danazol (Figure 1, a) for this study was purchased from Sigma-Aldrich. Carbamazepine (CBZ) and solid dispersion of CBZ with Soluplus® (CBZ-Soluplus HME, 15% CBZ load prepared through hot melt extrusion) were obtained from BASF Corporation (Tarrytown, NY).

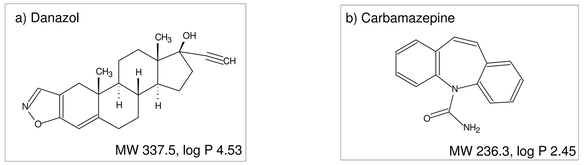

Figure 1. Structure and physicochemical properties of Danazol (a) and Carbamazepine (b), the model drugs used in this study.

The µFLUX™ device is an add-on option to the µDISS Profiler™ instrument (Pion Inc.) consisting of three pairs of temperature controlled side-by-side permeability chambers mounted on top of the stirring platform.

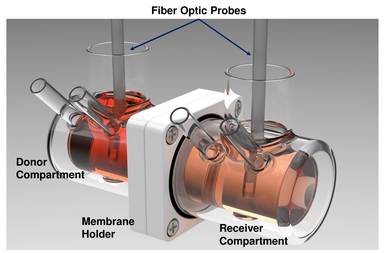

Figure 2. A fragment of the µFLUX apparatus showing a pair of the donor and receiver chambers. FO probes attached to the µDISS Profiler monitor concentrations in the donor (left) and receiver (right) compartments. The chambers can be separated by PAMPA, cell-based (Caco-2 or MDCK), dialysis, or other types of membranes mounted in the Membrane Holder.

Each pair (Figure 2) consists of a donor and an receiver compartment separated by a filter-supported GIT-optimized artificial membrane (Double-Sink™ PAMPA[2]). The donor compartment is filled with 10 - 13 mL of the media of interest. For this study the receiver compartment contained Acceptor Sink Buffer at pH 7.4 (ASB-7.4, Pion Inc). The integrated fiber-optic UV probes were positioned in the donor and receiver compartments allowing real time concentration monitoring in all chambers.

Fasted and fed state simulated intestinal fluids (FaSSIF and FeSSIF) were prepared by dissolving SIF powder in phosphate (pH 6.5, FaSSIF$_{blank}$) and acetate (pH 5.0, FeSSIF$_{blank}$) buffers following the protocol obtained from Biorelevant.com. All measurements were performed at ambient temperature.

Flux Measurements

Flux (J) of a drug through a biological membrane is defined as the amount of drug crossing a unit area perpendicular to its flow per unit time. In the one-dimension steady-state approximation it may be expressed through the effective permeability coefficient P_e and concentration $c(t)$ in the donor compartment as follows

$$J(t) = \frac{dm}{A \cdot dt} = P_e \cdot c(t) \qquad (1)$$

In the µFLUX device, the area A of the membrane is 0.78 cm^2 and the rate of appearance of material $\frac{dm}{dt}$ can be determined at any time point by continuously monitoring the concentration in the receiver compartment.

RESULTS AND DISCUSSION

Dissolution and Flux of Danazol in FeSSIF and FeSSIF$_{blank}$

Danazol is an extremely low-soluble compound and its concentration in FeSSIF$_{blank}$ (pH 5.0 acetate based buffer) reached saturation at ~0.3 µg/mL after about 1 hour of the dissolution-permeability experiment (Figure 3, a). This value is reasonably close to previously reported[3] value 0.9 ± 0.1 µg/mL measured at 37 ºC. Monitoring the concentration in the receiver compartment (ASB, pH 7.4) revealed zero-order permeation kinetics within the first 6 hours with concentration of Danazol slightly exceeding 0.2 µg/mL after this time period (Figure 3, b). Flux of Danazol through the 0.78 cm^2 Double-Sink™ PAMPA membrane was calculated to be 6.0·10^{-3} µg·min^{-1}·cm^{-2}.

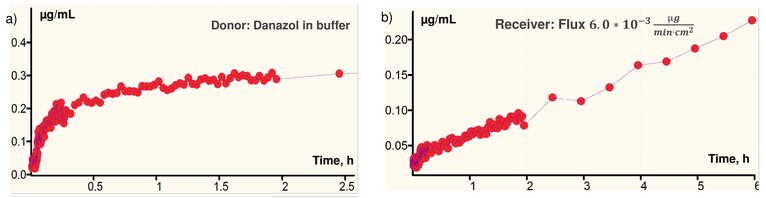

Figure 3. Dissolution (a) and appearance (b) of Danazol. Donor compartment (a): FeSSIF$_{blank}$; receiver compartment (b): ASB-7.4 buffer

Solubility of Danazol in FeSSIF was confirmed to be more than an order of magnitude higher than in the corresponding buffer[3] (Figure 4, a). After the initial 1.5 hours (dissolution time of Danazol in donor compartment) the flux in the receiver compartment was constant and could be determined by linear fitting of the concentration profile in the receiver compartment (Figure 4, b).

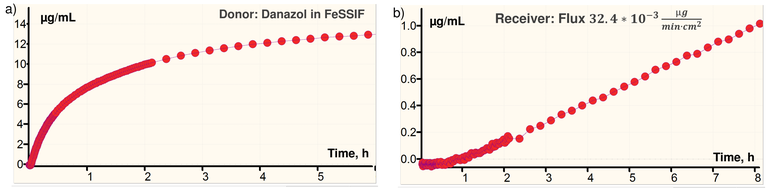

Figure 4. Same as Figure 3 except for the donor compartment (a) was filled with FeSSIF.

It is interesting to note, that although the total concentration of Danazol in the donor compartment was up to 40 times higher in the case of FeSSIF versus FeSSIF$_{blank}$ media, the flux increased only 5.4 fold. This indicates that solubility enhancement due to micelle formation does not lead to the same magnitude of absorption increase.

Supersaturation and Flux of CBZ and CBZ-Soluplus Dispersion

Supersaturation of pure CBZ and solid dispersion of CBZ with Soluplus has been studied previously[4]. Figure 5 a) shows the dissolution/precipitation profile of CBZ in pH 7.4 Prisma™ HT buffer (Pion Inc.) while Figure 5 b) represents the corresponding concentration profile of CBZ in the receiver compartment.

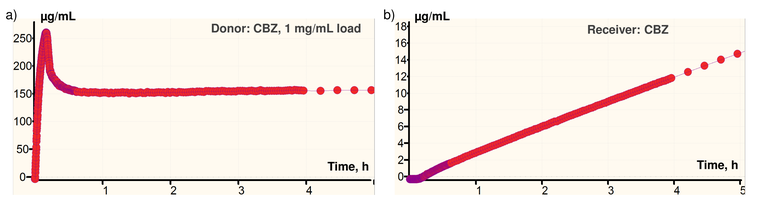

Figure 5. Dissolution, "spring" supersaturation and re-precipitation of CBZ in the pH 7.4 buffer (a) and appearance of CBZ in the receiver compartment (b).

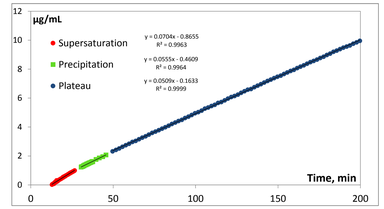

Figure 6. Linear fitting of concentration profile shown in Figure 5 b) for the flux values of CBZ corresponding to supersaturation, precipitation and equilibration regions of Figure 5 a).

The initial form of CBZ exceeded equilibrium solubility, but within 30 min it precipitated settling at about the 150 µg/mL solubility level. Figure 6 shows that despite quick re-precipitation, it was possible to detect differences between the initial flux of 1.2 µg·min^{-1}·cm^{-2} (supersaturation region) and the flux during precipitation (0.92 µg·min^{-1}·cm^{-2}) and when the concentration of CBZ reached equilibrium (0.85 µg·min^{-1}·cm^{-2}).

The CBZ-Soluplus dispersion was fully dissolved at the level of 1 mg/mL and stayed in this supersaturated form for about 4 hours followed by gradual re-precipitation (Figure 7, a). The concentration of CBZ after 16 hours still did not reach equilibrium and remained ~2.5 times higher than solubility of pure CBZ in the same buffer system.

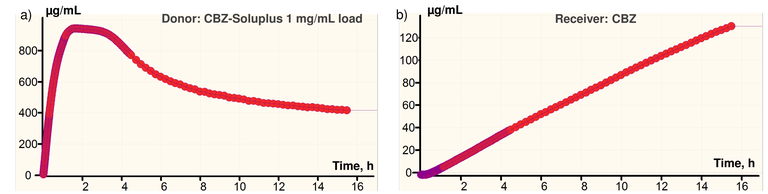

Figure 7. Same as Figure 5, but for CBZ-Soluplus HME showing "parachute" supersaturation behavior of CBZ in this case.

Flux of CBZ into the receiver compartment remained constant within a 0.5 – 4 h period and was ~3 times higher than for pure CBZ (2.7 µg·min^{-1}·cm^{-2}, Figures 7, b and 8). Following the onset of re-precipitation it decreased only slightly to 2.4 µg·min^{-1}·cm^{-2} remaining ~3 times higher than corresponding and ~2 times higher than the maximum flux of pure CBZ (Figure 8). Solubility of CBZ-Soluplus in donor was more than 6 times higher than one of CBZ.

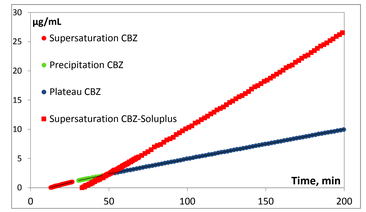

Figure 8. Comparison of CBZ flux from CBS-Soluplus HME (squares) and pure CBZ (circles) for the first 200 min of the assay.

CONCLUSIONS

In many cases, dissolution experiments alone cannot correctly predict the *in vivo* response to formulations due to the peculiar interplay of solubility and permeability in complex media.

Flux of Danazol in FeSSIF increased only 5.4 fold despite more than a 40 times increase in solubility over pure CBZ.

Analysis of dissolution, precipitation, and flux of CBZ indicates that effects of both supersaturation and complexation are present in the CBZ-Soluplus solution.

The µFLUX device extends the utility of *in situ* concentration monitoring to improved assessment of formulation on absorption potential and more realistic IVIVC modeling.

REFERENCES

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