FLUX Measurements using Pion µFLUX™ and MacroFLUX™ Devices

Introduction

Amount and the rate of absorption for the active pharmaceutical ingredient (API) to the blood circulation from the orally administered drug products is determined by the flux of API through the epithelial lining of the small intestine. The flux values would depend on the amount of the dissolved API available at the site of permeation as well as on the rate with which drug penetrates the membranes separating GIT from blood capillaries. The former quantity is governed by dissolution and solubility of API in the corresponding medium while the latter is determined by effective permeability of the compound through the biological membranes. Administering formulating API or a drug product will introduce formulation vehicles into the medium. These (non-active) additives are often designed to improve dissolution and solubility characteristics of the API. The effect of such formulations is commonly studied by comparing dissolution and solubility of API and its various formulations and selecting ones that produce highest improvement in these characteristics. However, formulation agents would also alter the effective permeability for drug compound in question [1]. Such effects are rarely studied and there is shortage of tools and methods that would allow quantitative assessment of formulation effect on permeability. On the other hand, it was demonstrated [2, 3], for example, that flux measurements provide more in-depth understanding of supersaturated systems than solute concentration measurements alone. One of the conclusions in [3] stated that there were flaws in using solute concentration alone in estimating supersaturation, and reaffirmed the use of flux measurements to understand supersaturated systems. Another study showed that flux measurements could be used for predicting drug-drug interactions with pH-modifying agents [4].

This technical note introduces devices that can be utilized for flux measurements in a systematic and reproducible manner. The small volume apparatus called µFLUX is compatible with Pion's mini-bath (MB-8) and can be used on various stages of formulation development when amount of API and/or its formulations is limited while many permutations of different formulation strategies have to be investigated. The other device MacroFLUX is an absorption chamber insert into USP 1 or 2 dissolution bath vessels. Both apparati would allow assessment of complex interplay between solubility, permeability and dissolution rate in formulation development and would provide valuable tools for *in vivo* predictive *in vitro* studies.

FLUX Measurements

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

$$J(t) = \frac{V dc_A(t)}{A \cdot dt} = P_e \cdot c_D(t) \qquad (1)$$

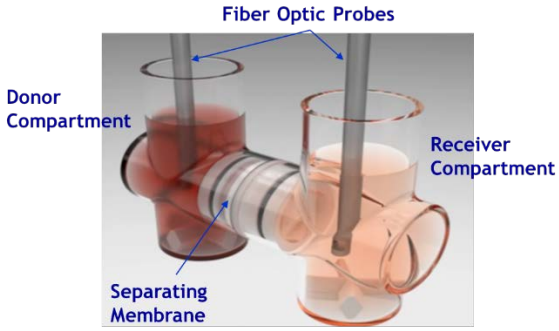
As evident from equation (1) above the flux can be measured from the slope dc_A/dt of concentration – time profile in the acceptor chamber ($c_A(t)$) multiplied by the volume in the receiver compartment (V)

and divided by the area of the membrane (*A*). It is also clear that flux measurement will reflect changes in both parameters—concentration or solubility in the donor and effective permeability.

It is interesting to note that even if concentration in the donor compartment cannot be measured for any analytical reasons (e.g., excessive turbidity or UV activity of formulation ingredients), the changing flux will indicate concentration changes in donor indirectly.

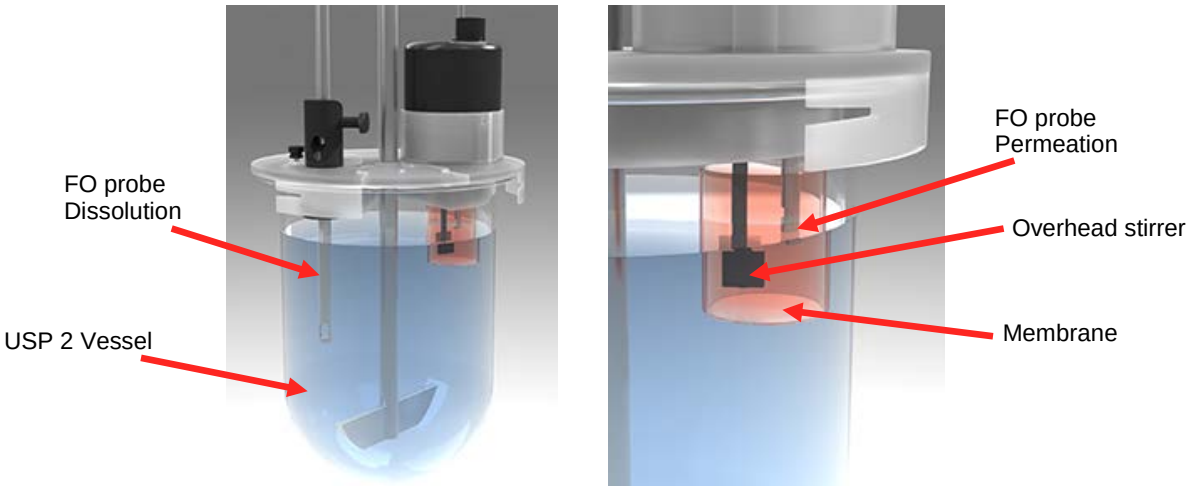
Figures 1 and 2 below show schematics of µFLUX and MacroFLUX devices that can be used for flux measurements at various stages of formulation development.

Figure 1. Schematic of µFLUX donor-acceptor pair separated by a membrane placed in the membrane holder. Fiber optic probes are shown inserted in the both compartments.



In the typical µFLUX experiment formulations are introduced to the donor compartment of µFLUX apparatus (Figure 1) containing 16–20 mL of dissolution medium as powders or suspensions. Receiver compartment contains the same volume of Acceptor Sin Buffer (ASB, Pion Inc.) that maintains pH 7.4 while having chemical scavenger ensuring sink conditions in the receiver chamber. Donor and acceptor compartments are divided by a lipophilic membrane (Double-Sink™ PAMPA model [5]) and concentrations are monitored using the µDISS Profiler™ (Pion Inc.).

Figure 2. MacroFLUX setup



A MacroFLUX device (Figure 2) consists of up to 6 cylindrical absorption chambers with 13–15 mL working volume and a filter supported artificial lipophilic membrane (Double-Sink PAMPA model [5]) with area 3.88 cm^2 attached to the bottom of the absorption chambers. These compartments are inserted into modified vessel covers of the dissolution bath (Erweka Model DT 126 light). Concentration monitoring in both dissolution and absorption chambers is enabled through fiber optic UV probes connected to the Rainbow instrument (Pion Inc.). Stirring in the absorption chamber is done using overhead stirrer bundled with measuring mini UV probe while the standard paddle of USP II apparatus provides stirring in the dissolution vessels.

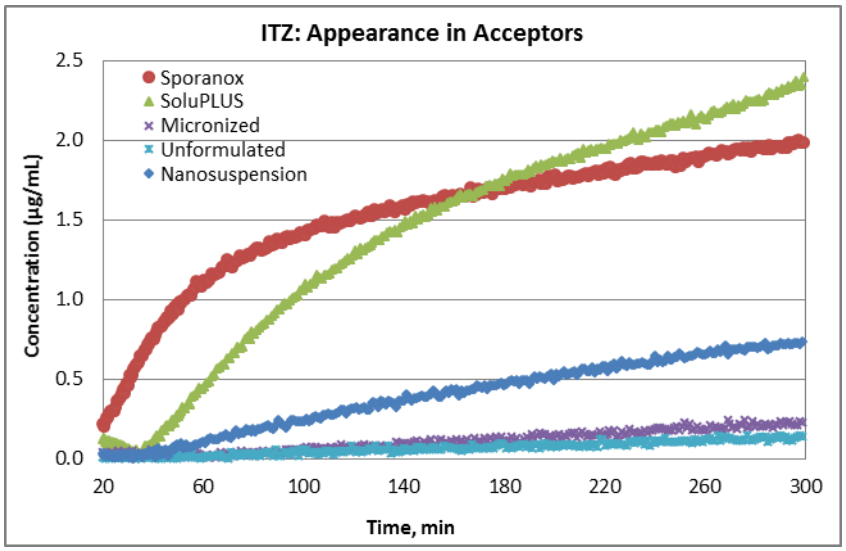
Some Applications of FLUX Measurements

Itraconazole Formulation Ranking

Nanoparticles of itraconazole (ITZ, 207 nm mean particle size) were prepared as 10 wt% suspensions in DI water with small amounts (< 3 wt%) of stabilizing excipients. Untreated (x_{50} = 17.8 µm) and micronized (x_{50} = 1.7 µm) powders of ITZ were suspended in the same media before the assay. Sporanox® solid dispersion commercial formulation and ITZ-Soluplus® solid dispersion extrudates [6] were assayed as milled and sieved powders. All formulations were introduced to the donor compartment of µFLUX apparatus containing 20 mL of FeSSIF at 0.4 mg/ml of ITZ. Temperature was set to 37 °C.

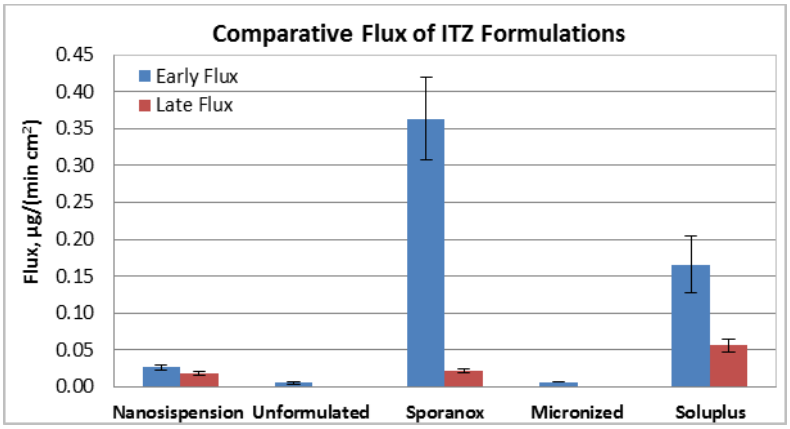
Concentration of ITZ in the receiver chambers was monitored with a dedicated blank pair (no ITZ) assessing integrity of the membrane and allowing additional correction for the background signal Figure 3). It was impossible to monitor concentration in the donor due to the excessive turbidity from undissolved ITZ.

Figure 3. Example of concentration-time profiles of ITZ in the receiver chambers of µFLUX system from different formulations of ITZ



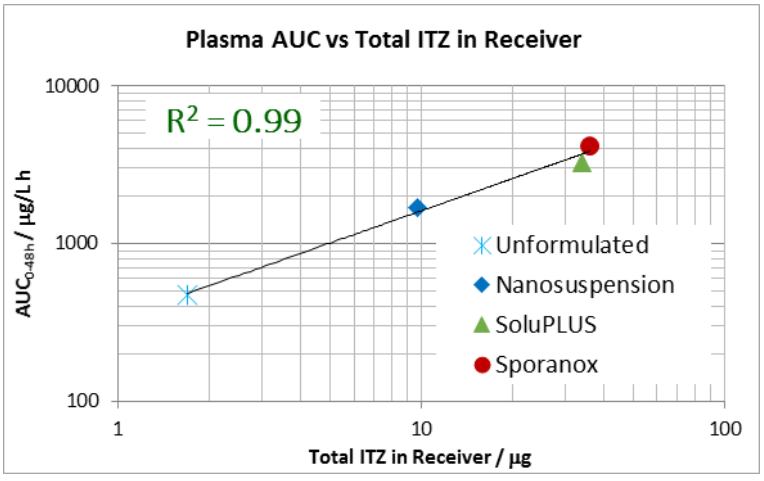
Flux values calculated from the slope of the concentration – time profiles for both unformulated and micronized ITZ did not change over the duration of the experiment (Figure 3, 4). Flux from other formulations was changing over time indicating the concentration change in the donor chamber. This phenomenon was also independently confirmed by solid form analysis [7].

Figure 4. Flux values at the beginning (blue) and at the end (brown) of the experiment. Error bars indicate SD from triplicate measurements.



Comparing with published PK data demonstrated great correlation between total amount of ITZ in the receiver chamber of µFLUX and AUC of plasma concentration profile [6] in animal studies (Figure 5).

Figure 5. AUC for ITZ concentration in plasma from animal data [6] versus total amount of ITZ in receiver from µFLUX after 240 min.



It was demonstrated that *in vitro* flux measurements using lipophilic artificial membranes could correctly reproduce rank order of rat PK results for different ITZ formulations. The drop in flux over time for solid dispersions could be backed by experimental indications of precipitation.

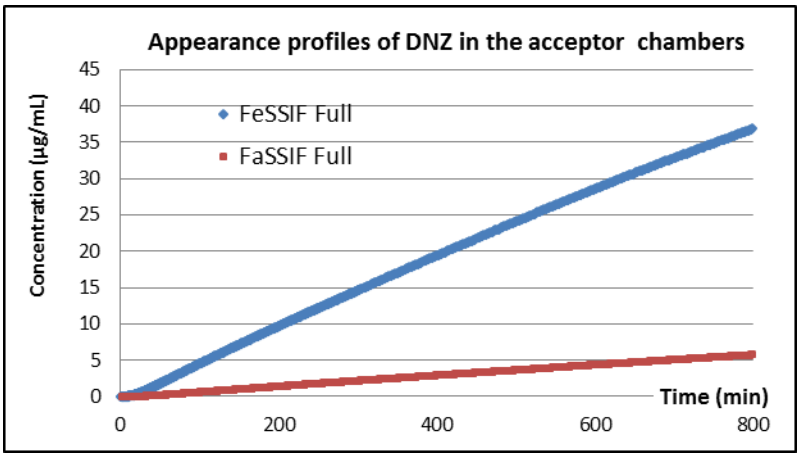
Food effect estimation

To predict positive or negative food effect on drug absorption the flux experiments were setup with donor compartment filled with FaSSIF or FeSSIF media to simulate fasted or fed intestinal conditions correspondingly. The ratio of the API flux values between receiver chambers paired with FeSSIF and FaSSIF media filled donors respectively was used to predict whether positive or negative food effect is expected for the API in question.

BCS class 2 drugs Danazol (DNZ, MW 337.5, non-ionizable in pH 2.0–9.0 range, logP 4.5) and Griseofulvin (GSF, MW 352.8, no ionizable groups, logP 2.2) were used as a model compounds for this study. API was delivered in the donor compartment of µFLUX apparatus containing 20 mL of FaSSIF or FeSSIF media at the loads 0.4 mg/mL for DNZ and 0.6 mg/mL for GSF. These loads correspond to the doses of 80 mg and 120 mg dissolved in 250 mL for DNZ and GSF respectively.

Maximum concentration of DNZ in the donor compartment containing FeSSIF was 30 µg/mL (7.5% dissolved) while in FaSSIF its concentration reached only 8 µg/mL (2% dissolved). Correspondingly the flux of DNZ from FeSSIF was 0.55 ± 0.03 µg $min^{-1}cm^{-2}$ comparing to 0.08 ± 0.02 µg $min^{-1}cm^{-2}$ from FaSSIF (Figure 6). Strong positive food effect (~3–4 fold) for DNZ was also reported for *in vivo* studies [8].

Figure 6. Concentration-time profiles of DNZ in the receiver chambers of µFLUX system (average of two replicates).



Concentration of GSF in FeSSIF (36 µg/mL) was twice as high as in FaSSIF (18 µg/mL). However, there was no differences in flux for GSF: 0.24 ± 0.03 µg $min^{-1}cm^{-2}$ and 0.23 ± 0.01 µg $min^{-1}cm^{-2}$ from the FaSSIF and FeSSIF respectively. Milder food effect (~1.7 times) was reported for GSF from *in vivo* studies [8].

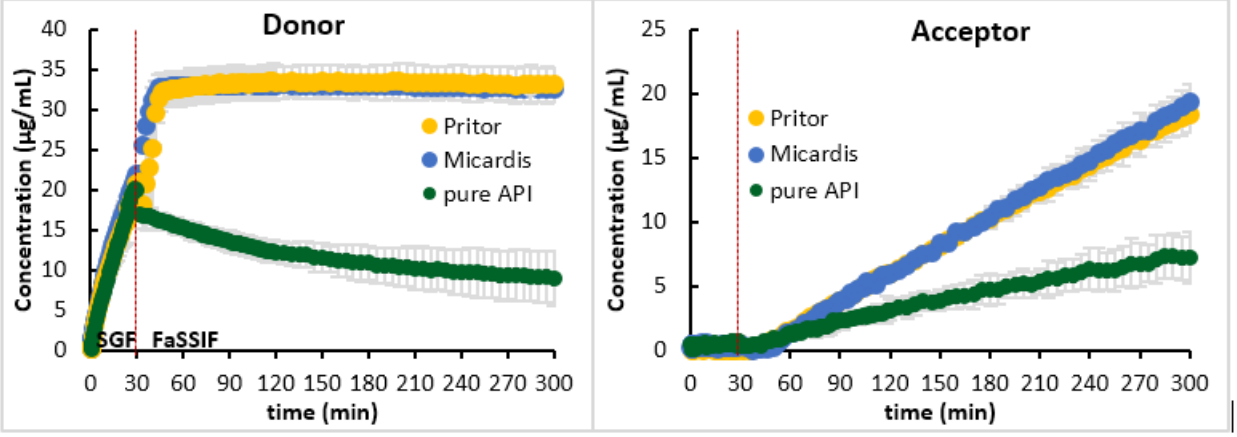
Bioequivalence study prediction

For generic drug development traditional (USP) dissolution tests have been used in the pharmaceutical industry to compare performance of different drug product formulations before or instead of conducting bioequivalence studies. Although dissolution tests provide a simple way of testing formulations, the *in vivo* predictive power of these tests is questionable. Namely, when a poorly water-soluble API is formulated to enhance its dissolution, additives, such as surfactants and polymers have an effect not only on dissolution profile, but also on flux through the membrane. This study illustrated the importance of incorporating the absorption chamber into USP II apparatus by using MacroFLUX device for better prediction of outcome of the bioequivalence studies.

Brand (Micardis®) and generic (Pritor™ and Actavis) formulations of Telmisartan, an antihypertensive drug, were tested using MacroFLUX. The experiment began in 850 mL at pH 1.6 simulating gastric conditions and then after 30 min the medium in the dissolution vessel was converted to FaSSIF by adding 212 mL of specially formulated concentrate containing SIF powder.

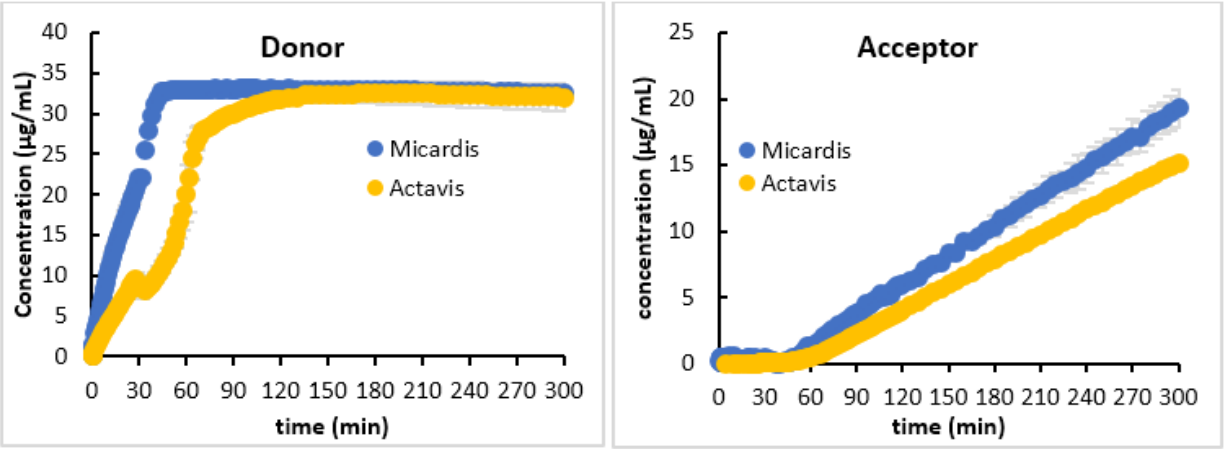
The dissolution and flux results of the Micardis and Pritor 40 mg tablets were compared (Figure 7). Both formulations showed slow release kinetics in SGF and instant dissolution after media conversion to FaSSIF with the final concentration around 35 µg/mL (more than 90% of the API dissolved). After the media change (time interval of 50-120 minutes) the flux through membrane was found to be 0.34 ± 0.03 µg/(cm^2*min) in case of the brand and 0.31 ± 0.01 µg/(cm^2*min) in case of the generic product. These findings were in line with *in vivo* studies where no significant difference was found between Micardis and Pritor formulations.

Figure 7. Dissolution profile (on the left) and appearance profile (on the right) of Telmisartan from Micardis (blue) and Pritor (yellow) and pure API (green). Red dashed line indicates the time when SGF was converted to FaSSIF.



The dissolution and flux results of the brand name (Micardis) and generic (Actavis) Telmisartan 40 mg tablets were also compared (Figure 8). Actavis showed a slower release kinetics than Micardis, though reached the same maximum concentration after 110 min. After media change the flux from the generic product was found to be only 71% of the flux of the brand name. This *in vitro* result showed excellent agreement with the *in vivo* data from bioequivalence studies [9], where the appearance rate or the drug in blood from Actavis was 72% of the rate from Micardis.

Figure 8. Dissolution profile (on the left), *in vitro* appearance profile (in the middle) and *in vivo* appearance (on the right) of Telmisartan from different formulations.



This example illustrates the *in vivo* predictive power of the simultaneous dissolution-absorption test using MacroFLUX apparatus.

Flux saturation phenomenon

It is evident from the Equation (1) that if permeability is constant the flux value is expected to be linearly proportional to the concentration of dissolved compound. This dependence was confirmed for various drugs including hydrophilic neutral compound like caffeine (Figure 9) as well as lipophilic neutral compound nifedipine (Figure 10).

Figure 9. Flux versus concentration in the donor for hydrophilic neutral compound caffeine. The data was obtained using disposable (plastic) µFLUX pairs.

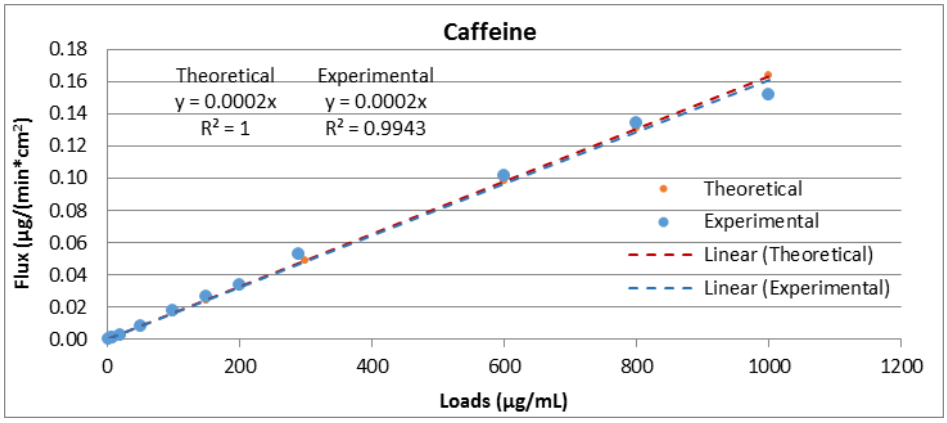
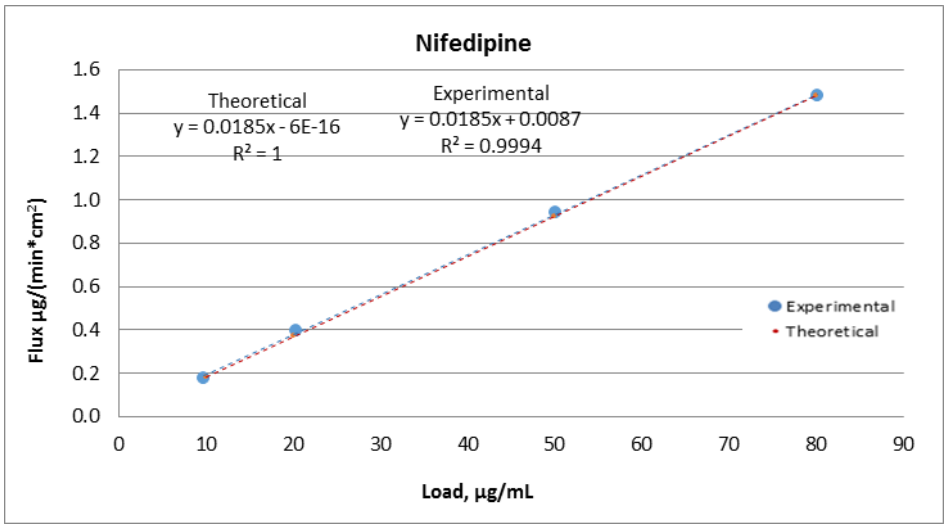
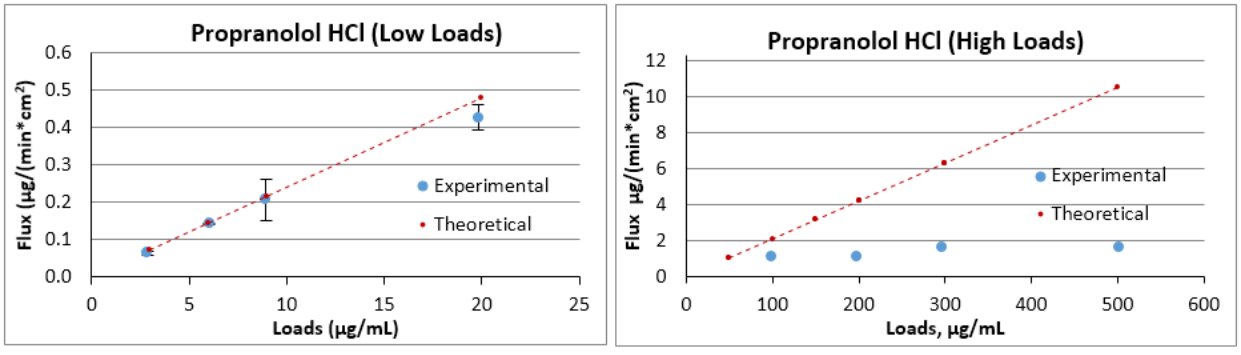


Figure 10. Flux versus concentration in donor for lipophilic neutral compound nifedipine. Higher concentration in the donor could not be reached due to exceeding the solubility of nifedipine in the donor medium. The data was obtained using glass µFLUX pairs.



An interesting phenomenon was noted recently when looking into unusual behavior of the flux versus load dependency for the basic lipophilic drug propranolol. Such class of compounds (pK_a ~ 9.0 or higher) are ionized at the biorelevant pH conditions (i.e. in the pH range 2.0–7.4) and therefore could be dissolved to relatively high concentrations. It was expected that flux values would be dependent on the load of the drug similarly to what is shown on Figures 9 and 10. However, it was discovered that after certain load the flux was not increasing or its increase was not proportionally to the increase in concentration. This behavior is presented on Figure 11 where for the relatively small loads of propranolol hydrochloride salt flux was proportional to the concentration of API, but at the larger concentrations such expected proportionality did not hold.

Figure 11. Flux versus concentration for lipophilic base propranolol (pK_a 9.5, logP 3.5). Flux is proportional to concentration for relatively small loads and reaches saturation between 20 and 100 µg/mL (right). Theoretical line on the right plot is continuation of the one on the left with assumption that flux would be proportional to concentration of propranolol.



It has been shown in multiple studies (e.g., [1, 5]) that only neutral form of compound can permeate (i.e. partition into) the Double-Sink PAMPA membrane. It means that on the donor/membrane interface the neutral form of the drug partitions to the membrane and in the donor medium the equilibrium between charged and un-charged species is re-established according to the pH of the medium and pK_a of the compound. For lipophilic compounds (especially lipophilic bases [5]) concentration in the membrane can be several order of magnitudes (~ 7 for propranolol) higher than concentration in the aqueous medium of donor compartment. Also, because of the huge donor/membrane volume ratio in the flux setups there is practically infinite pool of compound in the donor to partition to the membrane. It is plausible that due to solubility limit in the membrane at certain concentration in the donor there could be no more compound partitioning to the lipid. That means that flux through membrane would stop changing despite the increase of the donor concentration – i.e. membrane gets saturated. This hypothesis would explain why the flux of propranolol did not increase with increase of its concentration in the donor.

So far, the flux saturation effect has only been observed for the lipophilic high pK_a bases. This notion has to be kept in mind when this class of compounds is subjected to the comparative flux studies. Unchanged flux in these cases could indicate the saturation effect rather than lack of the formulation response.

The study is being continued to better understand the limitations of the flux saturation effect, its causes and to find a solution that would enable overcoming such limitations.

Conclusions

It has been demonstrated that flux assays allow investigation of complex formulations and can help building realistic PK predictions. Introduction of an absorption chamber into the USP apparatus can lead to more biorelevant dissolution studies.

While advantages of combining dissolution vessel with permeation chamber are clear, more investigation is required to better characterize compatibility of the artificial membrane against various excipients used in formulations and to understand limitations of the flux saturation phenomenon.

References

1. Avdeef A, Kansy M., Bendels S., Tsinman K. Absorption-excipient-pH classification gradient maps: Sparingly soluble drugs and the pH partition hypothesis. Eur. J. Pharm. Sci. 2008, 33, 29-41.
2. Raina S., et al. Enhancement and Limits in Drug Membrane Transport Using Supersaturated Solutions of Poorly Water-Soluble Drugs. J. Pharm. Sci., 2014, 103 (9), 2736–2748.
3. Raina S., et al. Impact of Solubilizing Additives on Supersaturation and Membrane Transport of Drugs. Pharm. Res. 2015, 32, 3350-3364.
4. Zhu A. Z. X., et. al. Utilizing In Vitro Dissolution-Permeation Chamber for the Quantitative Prediction of pH-Dependent Drug-Drug Interactions with Acid-Reducing Agents: a Comparison with Physiologically Based Pharmacokinetic Modeling. AAPS J., 2016, DOI: 10.1208/s12248-016-9972-4.

5. Avdeef A., Tsinman O. PAMPA—A drug absorption in vitro model. 13. Chemical selectivity due to membrane hydrogen bonding: In combo comparisons of HDM-, DOPC-, and DS-PAMPA models. Eur. J. Pharm Sci. 2006, 28 (1), 43-59.
6. Zhang, et al. Increased dissolution and oral absorption of itraconazole/Soluplus extrudate compared with itraconazole nanosuspension. Eur. J. Pharm. Biopharm., 2013, 85, 1285-1292.
7. Tsinman O., et. al. Differentiating Itraconazole Formulations Based on the Flux through Artificial Lipophilic Membrane. Poster presented at AAPS 2016 Annual Meeting. November 13 – 17, 2016, Denver, CO, USA.
8. K. Sugano. Biopharmaceutics modeling and simulations; Theory, Practice, Methods and Applications. John Wiley & Sons, Inc., 2012, 399p.
9. Public Assessment Report for Telmisartan Actavis. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/001168/human_med_001380.jsp&mid=WC0b01ac058001d124