Exploring the value of flux measurements in drug formulation and development:
Bioavailability beyond dissolution rate alone

Dissolution testing determines the speed with which an active pharmaceutical ingredient(s) (APIs) is released and solubilized from a formulated matrix. This process is a critical precursor to drug uptake into the bloodstream and dissolution testing in accordance with pharmacopeial specifications is routine for oral solid dosage (OSD) forms. However, *in vivo* the API must also transit biological membranes within the gastrointestinal (GI) tract to enter the bloodstream. Absorption of the API, just like dissolution, plays a critical role in determining bioavailability but is typically subject to less *in vitro* investigation during formulation development. This can be problematic when it comes to predicting the *in vivo* behavior of certain drugs, notably those designated BCS (Biopharmaceutics Classification System) Class 2.

The BCS Class 2 designation indicates a drug with low solubility and high permeability. Increasing solubility is a primary focus when it comes to formulating these drugs but unfortunately many solubilization strategies inhibit transit through the biological membranes. As a result, formulation changes that improve dissolution and solubility can have a minimal or even negative impact on absorption, bioavailability, and overall drug delivery rate. For drugs such as these more holistic *in vitro* testing is required to better support formulation development and minimize the time and money invested in *in vivo* trials.

Flux measurements allow formulators to navigate this issue. The flux of an API is essentially the transit rate of molecules from donor (formulated drug) to acceptor (bloodstream) and jointly accounts for dissolution rate, solubility, and permeability. With flux measurements, formulators can more effectively leverage *in vitro* testing to securely predict *in vivo* behavior, for drugs that are poorly modeled by dissolution testing alone. They can be used to screen excipients, to robustly predict the performance of fully formulated products, and to improve the likelihood of successfully demonstrating bioequivalence (BE), in generic development.

Pion has pioneered the use of flux measurements and their application in Absorption Driven Drug Formulation (ADDF), a relatively new concept based on using flux measurements to ensure that a drug product delivers the target absorption rate. Reference 1 provides a detailed description of ADDF and presents case study data demonstrating its value in generic formulation development. Here we take a more general look at the application of the Pion portfolio of products for flux measurements, showcasing their ability to address specific issues at various stages of the formulation process. Case study data demonstrate the utility of flux measurements for excipients screening, to rank alternative formulation, to predict the likely outcome of *in vivo* PK (pharmacokinetic) trials and to assess the impact of fed state on drug absorption.

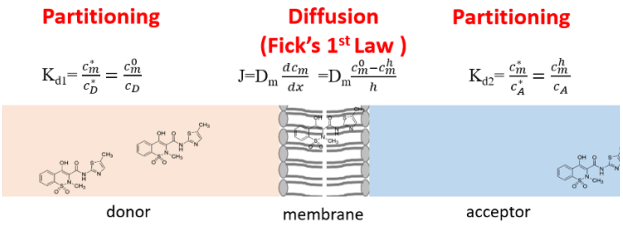
FOCUSING ON FLUX

Closer examination of what happens to an OSD product *in vivo* is helpful in elucidating what is meant by flux and the relevance of measurement. In vivo, solubilized API reaches the bloodstream via absorption across the biological membranes of the GI tract. Flux quantifies this absorption process and is mathematically defined as the net number of moles crossing unit area of membrane per unit time, typically moles or $mg.min^{-1}.cm^{-2}$:

$$Flux \quad J = \frac{1}{A}\frac{dM}{dt} = \frac{V_A}{A}\frac{dc_A}{dt}$$

Where: A is the surface area over which flux is occurring (cm^2), V_A is the volume of the system (cm^3 and c_A is the concentration of API mg/cm^3.

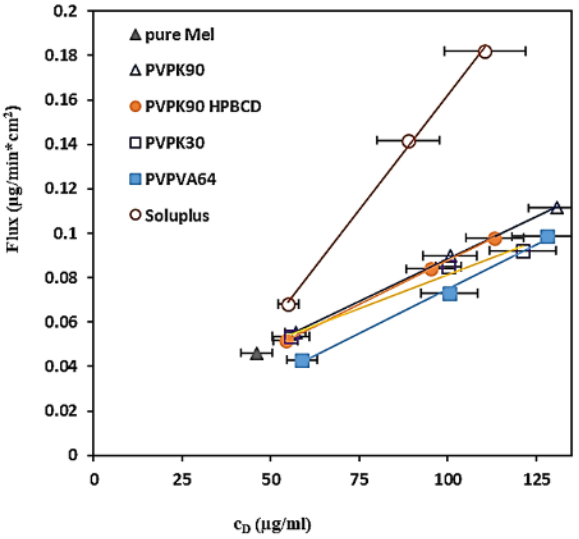
A permeation cell provides a useful model for the analysis of *in vivo* absorption (see figure 1). Dissolution releases API which partitions between the aqueous media in the donor compartment and the membrane exposed to it. Diffusion across the membrane transports API to the acceptor compartment where again there is partitioning between the aqueous media and membrane. Modeling these processes with partitioning coefficients, Fick's first law, and assuming constant solubility in the donor and acceptor media produces the simple relationship shown. Flux is related to permeability and the concentration driving force across the membrane which in many instances can be simplified to concentration in the donor cell.



$$J = P_e(c_D - c_A) \cong P_e c_D$$

Figure 1: A schematic showing absorption through a membrane in a permeation cell. Flux is dependent on permeability and the concentration gradient across the membrane.

If permeability is constant, then this equation indicates that concentration in the donor compartment will be the sole factor influencing flux, in which case dissolution/ solubility data will provide a secure basis for formulation comparison. Figure 2 shows experimental measurements of flux as a function of donor concentration for various meloxicam formulations[1]. As expected, flux is directly proportional to donor concentration in each case. However, the gradient of these plots varies from one formulation to another. The relationship between flux and donor concentration depends on the excipients present suggesting they change not only solubility but also the apparent permeability of the drug. At any given donor concentration flux is formulation dependent.



J= flux (µg/min*cm²min*cm²)
Pe= permeability (cm/sec)
c_D= donor concentration (µg/mL)
c_A= acceptor concentration(µg/mL)

Figure 2: Flux correlates directly with donor concentration for each meloxicam formulation[1], but the gradient of the plot is different for different formulations, highlighting the potential limitations of relying solely on dissolution data for formulation development.

Class 2 drugs call for the application of novel formulation strategies to improve solubility. Solubilizing agents such as surfactants, lipids, cyclodextrins or co-solvents all have potential but there is both *in vitro* and *in vivo* evidence to suggest that gains in solubility typically come at the price of loss in apparent permeability, a phenomenon referred to as the solubility-permeability interplay [2]. Strategies that are successful in improving solubility, may therefore ultimately have no beneficial impact on drug delivery.

Direct measurements of flux, as shown in Figure 2, help formulators to navigate this effect, since they capture the net effect of such changes. These measurements are therefore highly complementary to conventional dissolution testing for drugs that are particularly challenging to formulate.

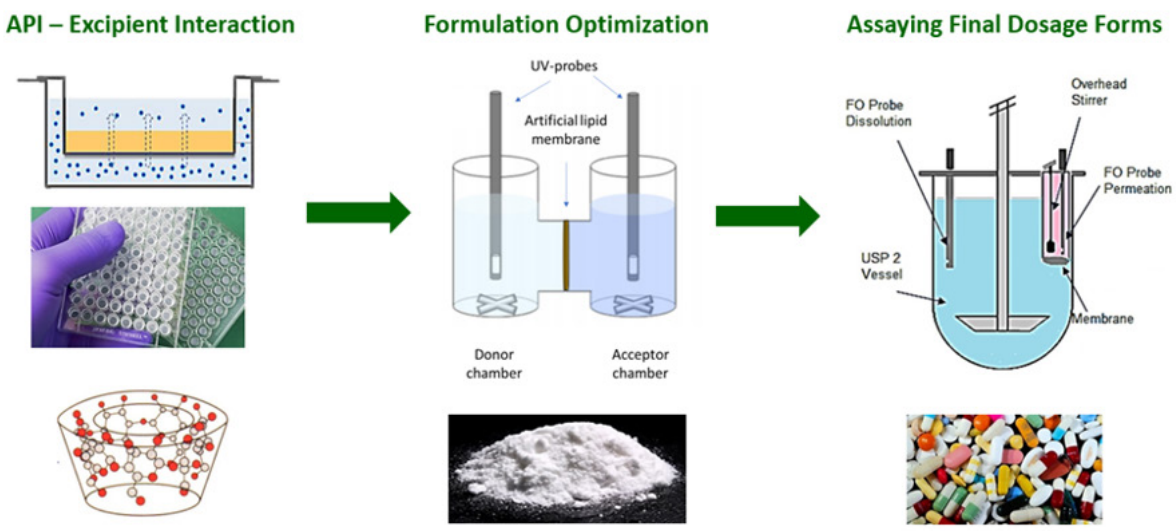


Figure 3: Pion offers solutions for flux assay for application from preformulation through to QC, left to right: PAMPA, Micro-FLUX and Bio/Macro-FLUX.

MEASURING FLUX

Pion has developed a range of solutions that enable flux (or dissolution-permeation) assays at each stage of formulation to augment the information accessible via routine dissolution testing and provide a more robust platform for product development. Figure 3 shows schematics of the systems available and their alignment with formulation workflows.

PAMPA (Parallel Artificial Membrane Permeability Assay - figure 3, left) is a 96 well plate-based system for high throughput preformulation excipient screening. Membranes are selected on the basis of their ability to mimic absorption in the GI tract to make these simple assays in biorelevant media as representative as possible, for comparative flux assessments with minimal sample volumes. PAMPA is a valuable tool for the rapid assessment of API-excipient interactions and the net effect of an excipient, helping to identify those that lead to an overall improvement in flux. It aids initial excipient selection and later in formulation supports better prediction and management of the impact of changes.

MicroFLUX (figure 3 – middle) is a small volume flux apparatus (donor and acceptor volumes are typically 16 – 20 ml) for the characterization of more complex formulation samples. Using biorelevant media it enables assays under conditions mimicking either the fasted or fed state for a wide range of liquid or solid samples, from granules and solid dispersions to emulsions and suspensions. Continuous concentration monitoring in both compartments provides detailed insight into both dissolution and permeation. MicroFLUX is an efficient solution for detailed assessment of the effects of an individual excipient, of changes in excipient concentration and for the investigation of alternative formulation strategies.

MacroFLUX (figure 3 – right) integrates an absorption chamber with permeation membrane into a standard 900 mL USP (US Pharmacopoeia) Type II apparatus for dissolution testing enabling large scale flux assays for finished OSD forms. Real-time API concentration measurement in the donor and acceptor compartments (as in MicroFLUX) provides detailed insight into tablet/capsule disintegration, dissolution, solubility, and permeability. **BioFLUX** is strictly comparable but has a smaller, more biorelevant donor volume of 200 – 250 ml enabling more representative studies of bio-performance. Both MacroFLUX and BioFLUX are valuable for product optimization, for batch-to-batch comparability studies and for bioequivalence (BE) testing - in lifecycle management, for product extension, and crucially, for generic product development.

The following case studies illustrate the information these systems provide and its value in predicting the outcome of *in vivo* studies.

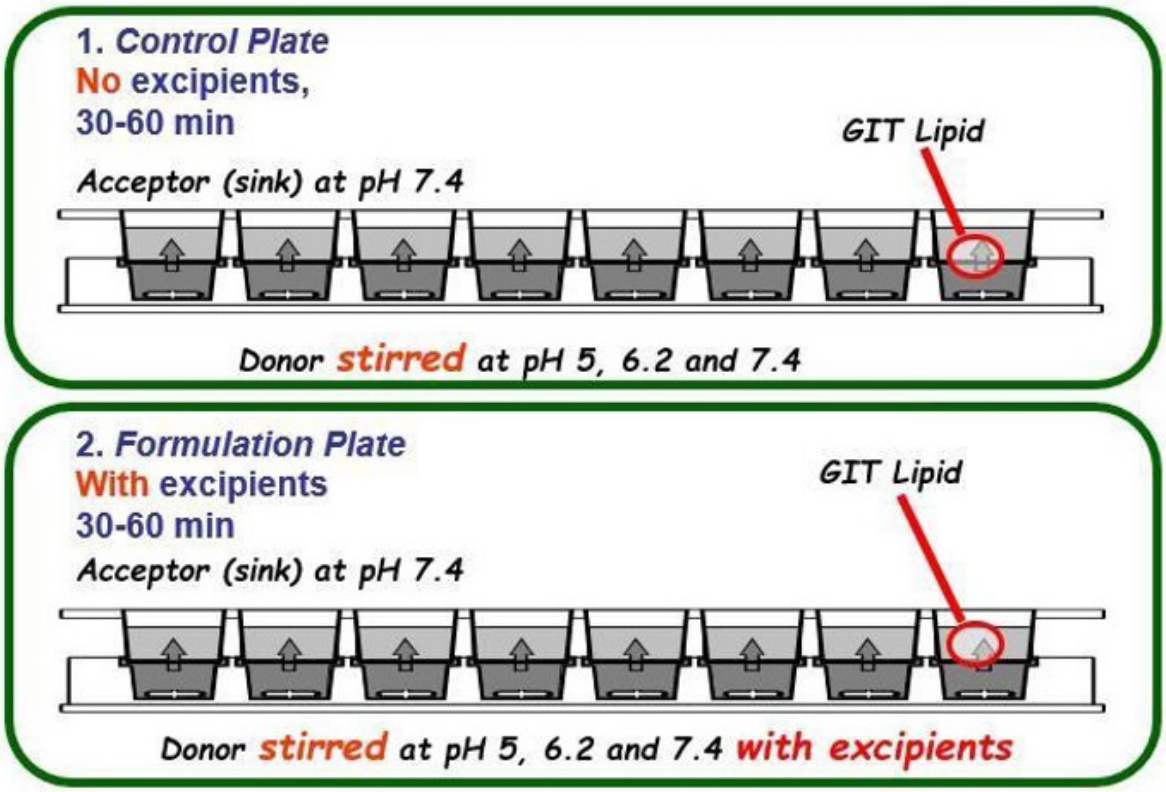


Figure 4: The PAMPA platform enables a simple flux assay for the assessment of excipient effect as evidenced in this experimental set-up for study of the interactions of different excipients with tamoxifen.

A study was carried out[3] using a PAMPA system to compare alternative excipients for tamoxifen, a drug used for the prevention and treatment of breast cancer. Figure 4 shows a schematic of the experimental set-up. Solid API samples are placed in the stirred bottom compartment of the plate. Donor media is added, with or without excipient, and the top plate, containing membrane coated with a GI tract lipid mixture, and acceptor media are placed on top. After a defined incubation period the plates are separated and the amount of API in the acceptor compartment is quantified by UV absorption. Tests were carried out using five different excipients: sodium taurocholate (NaTC)), which was tested at two concentrations, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), polyethylene glycol (PG), n-methylpyrrolidone, and Hypromellose (HP).

Comparing UV spectra in the acceptor plate (see figure 5) highlights which excipients lead to flux enhancement, since these show higher absorbance, notably at shorter wavelengths. Both 15 mM taurocholic acid and HP-β-CD show substantial flux enhancement at a pH of 7.4, though interestingly at a lower concentration taurocholic acid has a negative effect on flux, illustrating the importance of studying concentration effects. 1% HP also has a negative effect on flux while equivalent levels of n-methylpyrrolidone and PG have negligible impact, positive or negative. The results clearly demonstrate the ability of the system to differentiate the excipients with respect to flux enhancement and provide useful guidance for selection.

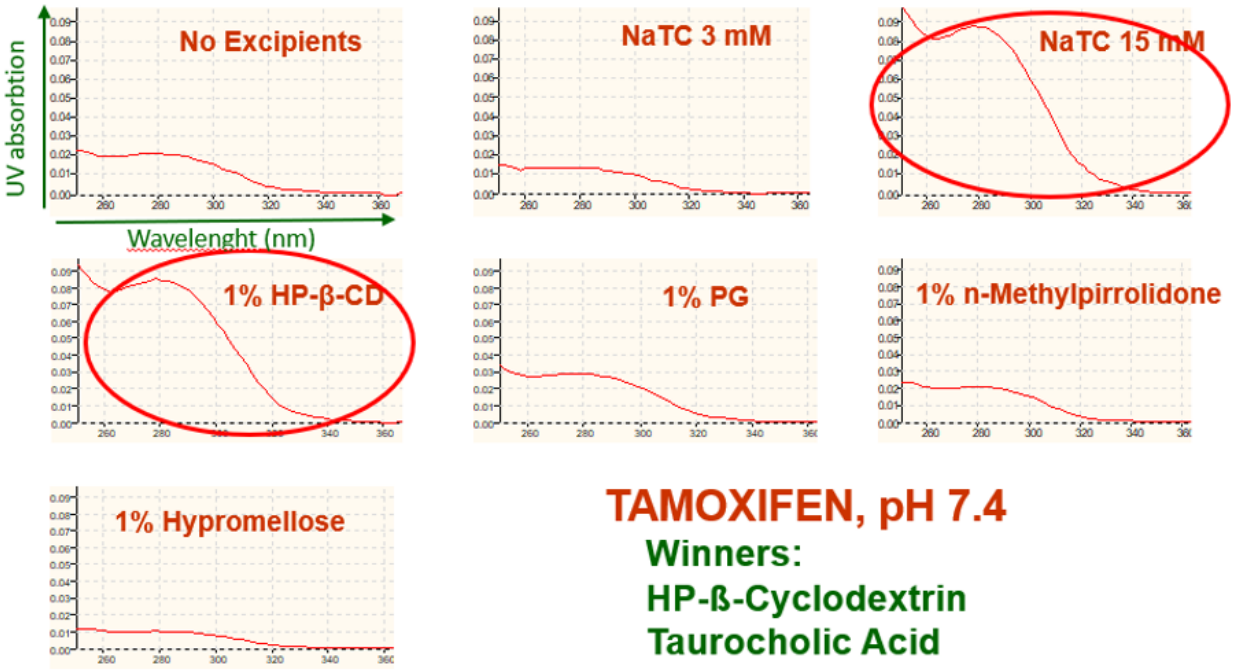


Figure 5: High-throughput flux assays readily identify the flux enhancement capabilities of sodium taurocholate and HP-β-CD relative to alternative excipients (or an absence of excipient).

CASE STUDY 2: RANKING THE PERFORMANCE OF ITRACONAZOLE FORMULATIONS

Assays were carried out using a MicroFLUX system to rank the dissolution-permeation performance of alternative formulations containing itraconazole, an anti-fungal medication[4]. Itraconazole was formulated in crystalline form – micronized powder and nanosuspension – and as an amorphous solid dispersion with Soluplus, a commercially available polymeric excipient. Testing was also carried out with Product A (Janssen Pharamceutica, Beerse, Belgium), a commercially available amorphous solid dispersion formulation of itraconazole sold in capsule form, and with the untreated API. The results were compared with PK data to determine whether the assay provided a reliable indication of *in vivo* performance.

Dissolution-permeation assay of ITRA containing formulations

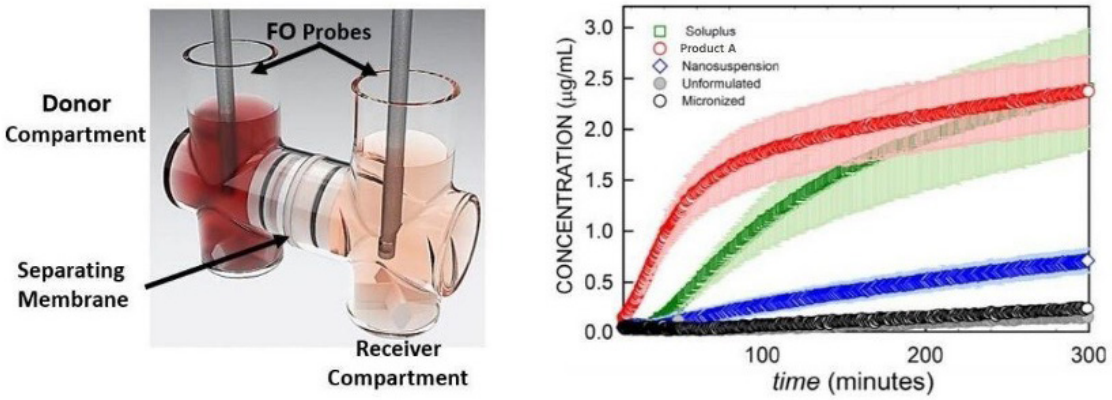


Figure 6: Itraconazole containing formulations can be successfully differentiated using a MicroFLUX side-by-side diffusion cell, with the two amorphous solid dispersions delivering the highest levels of flux.

Dissolution-permeation assay of ITRA containing formulations

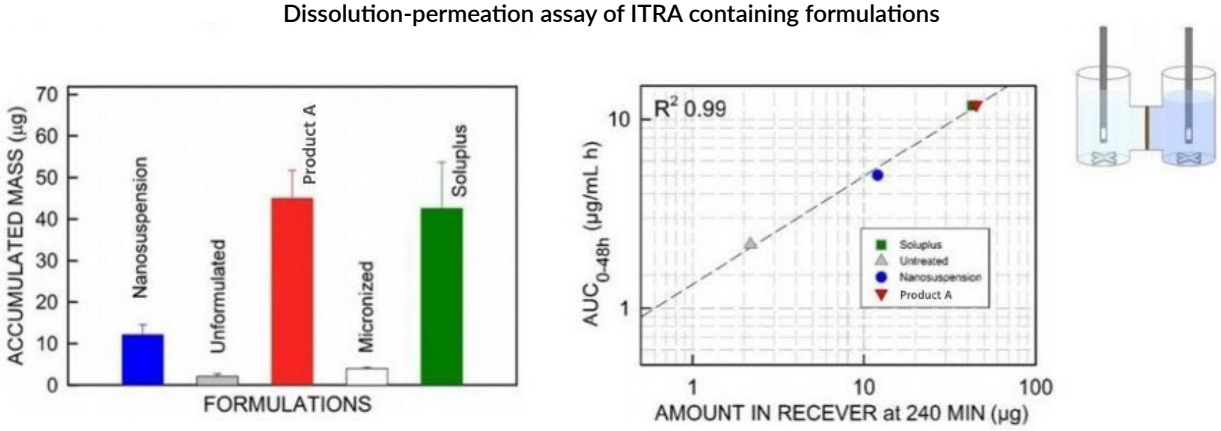


Figure 7: Close agreement between dissolution-permeation assays and in vivo PK data demonstrate the value of MicroFLUX measurements for the assessment of formulation performance.

Figure 6 shows the apparatus used and the results generated in terms of concentration build-up in the acceptor chamber. The donor chamber was filled with FeSSIF media (Fed State Simulated Intestinal Fluid media) due to the recommendation that Itraconoazole is taken after a meal, while the acceptor compartment was maintained at a pH of 7.4, representative of the blood stream. The separating membrane was coated with a GI tract lipid mixture to model absorption as representatively as possible.

The results show that formulation as an amorphous solid dispersion produces significantly higher flux than is observed with either of the crystalline forms. The commercial Product A formulation exhibits the highest initial flux but after about 2.5 hours the performance of the two amorphous formulations converges.

Figure 7 (left) shows accumulated mass data for the itraconazole in the acceptor compartment after 240 minutes. Though the permeation profiles of the two solid dispersions are different over this timeframe the accumulated mass of drug delivered is closely similar. The nanosuspension results in a significantly lower accumulated mass, with the unformulated and micronized itraconzole exhibiting the worst performance. Comparing this ranking with *in vivo* rodent PK data, AUC (area under the curve) results, demonstrates close correlation with *in vivo* performance. This study therefore clearly illustrates the value of the MicroFLUX in generating data for the assessment of alternative formulations, and for investigating equivalence in a test product, as part of generic product development.

CASE STUDY 3: PREDICTING THE RISK OF FAILURE IN BE STUDIES.

Studies were carried out to determine whether flux measurements could be used to predict the likelihood of success in BE studies for generic formulations of telmisartan, a poorly water-soluble drug used to treat high blood pressure[5]. A MacroFLUX apparatus was used to assess Drug 1, the branded/reference product, along with commercially available generics from “Company A”, “Company B”, “Company C”, and “Company D” (company names redacted). This OSD form is specified for administration in the fasted state so the dissolution vessel of the MacroFLUX was initially filled with simulated gastric fluid (SGF) with a pH of 1.6; a media change after 30 minutes to FaSSIF media (Fasted State Simulated Intestinal Fluid media) shifted the pH to 6.5. The absorption chamber was maintained at a pH of 7.4.

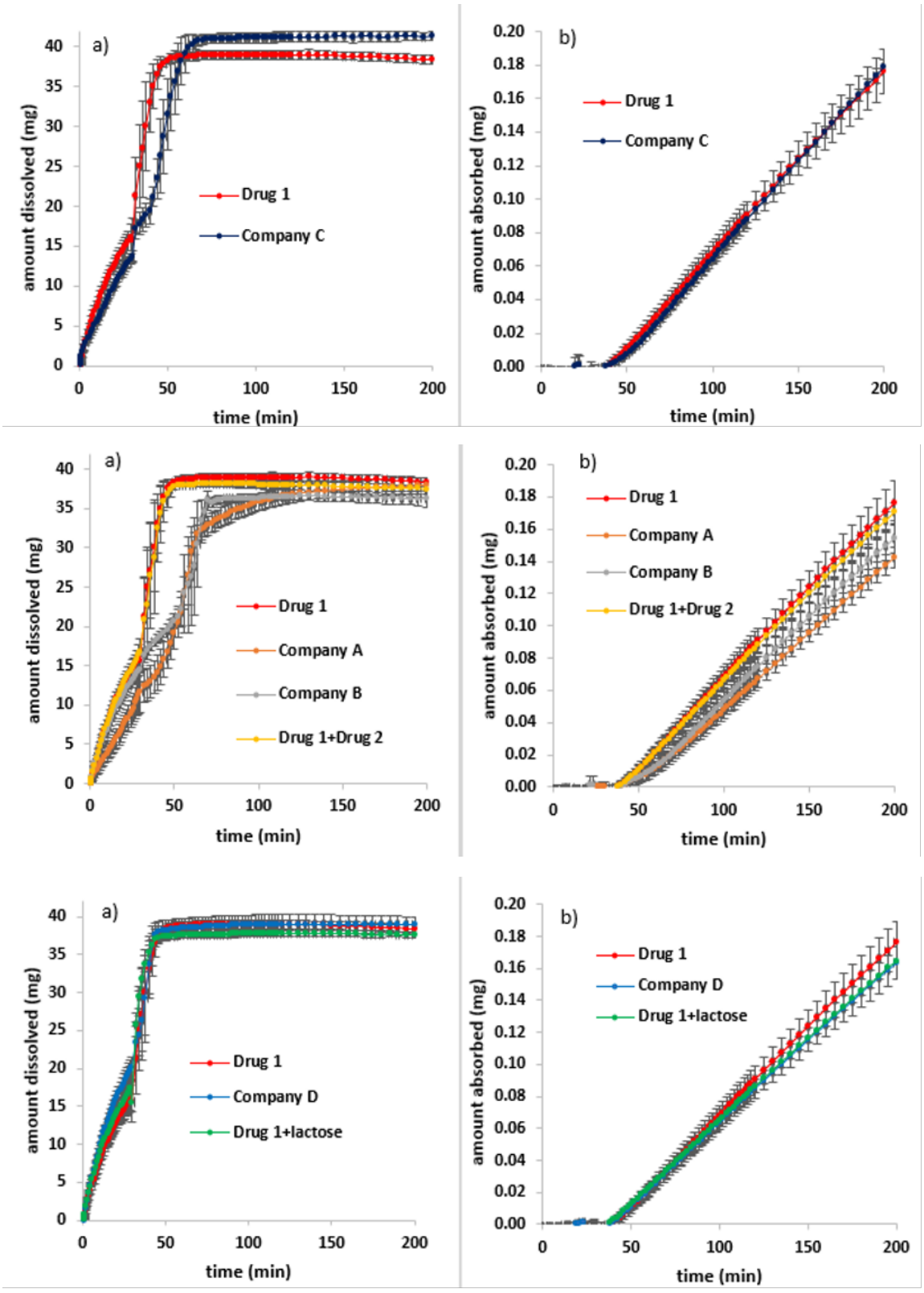


Figure 8: Data measured with the MacroFLUX differentiates generic products in terms of dissolution and absorption, providing robust data for the prediction of BE.

Figure 8 shows dissolution and acceptance/absorption profiles for each of the products. Company C generic (top, figure 8) produces a similar dissolution profile to the reference product exhibiting slow-release kinetics in SGF and accelerated dissolution following a switch to FaSSIF. The absorption profiles of Company C and Drug 1 products are extremely similar with no API crossing the membrane until the media conversion.

In this study, flux ratios and associated 90% confidence intervals were determined from log-transformed fluxes and used to predict the likelihood of BE acceptance on the basis that the 90% confidence intervals must fall within 80 – 125% of the reference, as in *in vivo* studies. For Company C product flux ratios were a near perfect match and the confidence intervals fell well within this range suggesting that this generic would deliver *in vivo* BE, as results confirmed.

Data for Company D generic (middle, figure 8) show closely comparable dissolution performance. Company D formulation contains lactose monohydrate in place of the sorbitol filler used in the reference product and dissolution testing of the reference product in the presence of lactose was carried out for this reason. Flux ratios for Company D generic were found to be well within the required confidence limits (see table 1) indicating a high likelihood of BE.

Data for the Company A and Company B generics (bottom, figure 8) show that these products have markedly delayed dissolution, relative to the reference product, notably after the media change, though similar maximum concentrations are reached after a period of around 2 hours. Absorption profiles show that Drug 1 delivers faster membrane transport than either generic, but especially Company A. Company A uses a mannitol filler, and though this may be a reason for the flux decrease it is clear from the additional testing done that other factors must also be influential; the observed difference cannot be attributed solely to the presence of mannitol.

Table 1 summarizes the measured data. From the flux ratios, BE acceptance would be predicted for all four generics, however, in the case of Company A the lower confidence interval fell just outside the 80% limit, suggesting borderline performance. *In vivo* data for this generic showed a similar pattern. The public assessment report records that the formulation was on the border line of the acceptance range and that the confidence interval most probably did not fall within the range because of high standard deviation in the *in vivo* results. This study therefore provides clear evidence of the value of flux measurements in predicting the likelihood of BE acceptance.

Name	Characteristic Filler	Flux ratio (%)	Lower 90 % CI in %	Upper 90 % CI in %	Prediction based on *in vitro* result	*in vivo* result of bioequivalence study	***In vitro* prediction matches *in vivo* results**
Company A	Drug 2	86.6	79.2	84.2	Borderline of acceptance	Borderline of acceptance 76 - 95	✓
Company B	Drug 2	92.1	85.7	99.4	Going to be accepted	Accepted	✓
Company C	Drug 3	100.1	97.3	103	Going to be accepted	Accepted 82 - 109	✓
Company D	Lactose monohydrate	91.4	87.3	95.8	Going to be accepted	Accepted 99 - 119	✓

Table 1: Comparing in vitro and in vivo data demonstrates the value of flux measurements for prediction of the likelihood of generic drugs succeeding in in vivo BE studies. From Reference 5.

Studies were carried out to determine whether flux measurements could be used to predict the effect of fed state on the performance of three different itraconazole formulations[6]. A BioFLUX apparatus was used to measure fraction absorption ratios (F_a - fed/fasted) for Product A solution (Janssen Pharmaceutica), which has shown higher bioavailability in the fasted state, Product A capsules (Janssen Pharmaceutica), which are supposed to be taken after a meal, and Product B capsules, a novel amorphous solid dispersion formulation.

For fasted state experiments the formulations were added to 200 mL of pH 1.6 buffer to simulate gastric conditions. The acceptor and donor compartments were connected 30 minutes into the experiment as the media in the dissolution vessel was changed to FaSSIF (through the addition of 50 mL of formulated concentrate). For fed state assays formulations were added to 250 mL of FeSSIF, no media change was required, and the acceptor and donor compartments were connected from the outset. An F_a ratio less than 1 reflects a negative food effect, while an F_a ratio greater than 1 reflects a positive food effect.

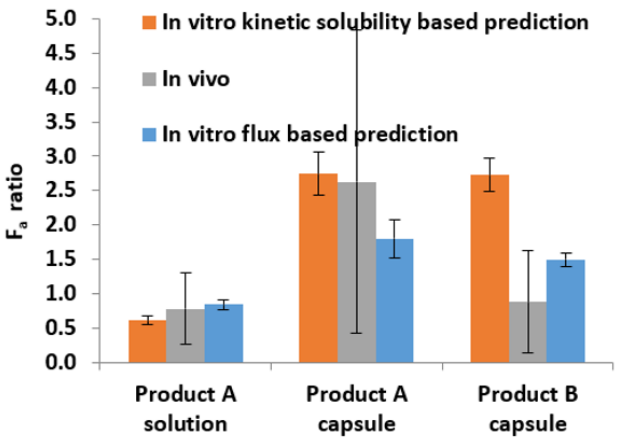


Figure 9: Data measured with BioFLUX captures observed differences in the *in vivo* performance of the formulations associated with fed state.

Figure 9 shows the F_a ratio values measured via flux experiments and how they compare with *in vivo* data (available from published literature) and predictions based on *in vitro* solubility measurements (which were also measured as part of the study – see reference 6 for further details). For Product A solution flux measurements predict a F_a ratio of less than 1 and a negative food effect, a result consistent with *in vivo* data and the recommendation of administration prior to a meal. In contrast, the F_a ratio derived for Product A capsules is above 1 showing a positive food effect, which is again consistent with *in vivo* data, and the advice for administration after a meal. For Product B capsules manufacturers have claimed a reduced food effect compared to the Product A capsule and the flux measurements support this, though a positive food effect is still observed. Overall, the flux measurements show good consistency with the *in vivo* data, ranking the food effect associated with the formulations in the same order; solubility data based prediction, in contrast, does not. Here then there is clear evidence of the value of flux measurements in predicting food effects in order to optimize therapeutic efficacy.

IN CONCLUSION

For certain drugs, notably BCS Class 2, an over-reliance on dissolution testing, in isolation, can be sub-optimal to efficient progress. The ADDF concept pioneered by Pion focuses on flux, and the use of assays that simultaneously measure dissolution and permeability to assess the net impact of formulation changes. Flux measurements allow formulators to identify the excipients and formulation strategies that are most likely to deliver the bioavailability and drug delivery performance required, rather than simply dissolution and solubility equivalence. Crucially flux measurements can be valuable indicators of the likelihood of BE acceptance, making them an extremely useful *in vitro* tool for generic developers. Using the ADDF concept to supplement conventional dissolution testing formulators can accelerate both new and generic products to market, even when tackling some of the toughest drugs.

REFERENCES

The real first is missing.

1. Kadar S. et al. 'Flux-Based Formulation Development—A Proof of Concept Study' *AAPS Journal* DOI: 10.1208/s12248-021-00668-9 (2022)
2. Borbás E. et al. 'Investigation and mathematical description of the real driving force of passive transport of drug molecules from supersaturated solutions.' *Mol. Pharm.* 13, 3816–3826 (2016).
3. Dahan, A. et al. 'The solubility-permeability interplay and oral drug formulation design: two heads are better than one.' *Adv. Drug Deliv. Rev.* 101, 99–107 (2016)
4. Tsinman, K. et al. 'Novel *in vitro* PK (non-animal PK) Assay for Excipient/Vehicle Formulation Studies.' AAPS 2008
5. Tsinman, K. et al. 'Ranking itraconazole formulations based on the flux through artificial lipophilic membrane.' *Pharm. Res.* 35, 1–13 (2018)
6. Borbás E, et al. 'The effect of formulation additives on in vitro dissolution-absorption profile and *in vivo* bioavailability of telmisartan from brand and generic formulations.' *Eur J Pharm Sci.* 114(1):310–7 (2018).
7. Borbás E, et al. 'Prediction of bioequivalence and food effect using flux and solubility-based methods.' *Mol Pharmaceutics*. DOI: 10.1021/acs.molpharmaceut.9b00406

Pion

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