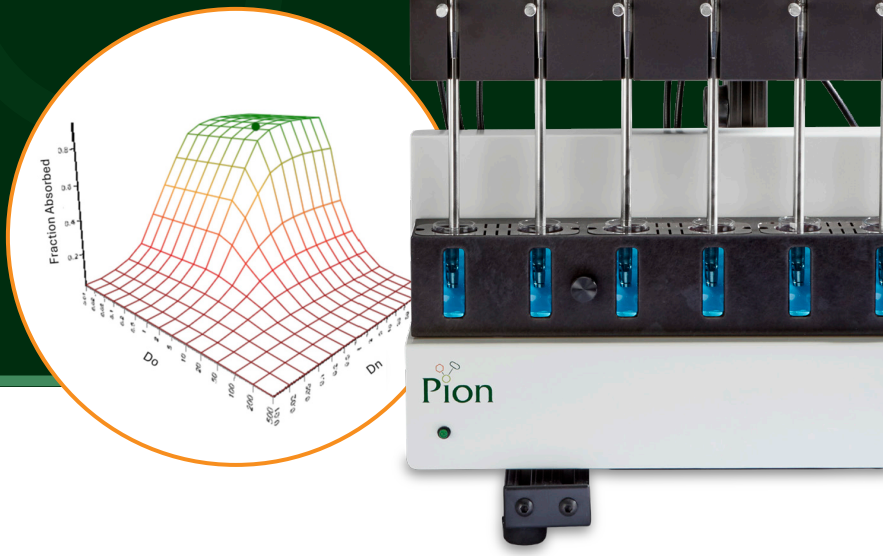
APPLICATION NOTE

Improving Absorption Prediction Using Integrated Dissolution–Permeation Testing and Mechanistic *In Silico* Modeling



Pion's MicroFLUX with Predictor Fraction Absorbed graph

Limited aqueous solubility remains a major barrier to optimized bioavailability of oral drugs, particularly for compounds classified as Biopharmaceutics Classification System (BCS) Class II. For these molecules, formulation-driven enhancements in solubility and dissolution are often required for driving oral absorption and can be essential for achieving the desired *in vivo* effect.

Numerous formulation strategies have been developed to improve solubility and dissolution behavior, including the use of amorphous solid dispersions and particle size reduction techniques. While these strategies can improve apparent solubility, such improvements do not consistently translate into predictable *in vivo* performance, highlighting the shortcomings of conventional dissolution testing as a standalone predictor of clinical outcomes.

Dissolution–permeation assays reveal the interplay among solubilization, supersaturation, precipitation, and intestinal permeability—key drivers of oral absorption that single-phase dissolution methods fail to capture. Biorelevant data captured from *in vitro* dissolution-permeation testing can be integrated with mechanistic *in silico* absorption models to improve prediction of oral *in vivo* drug performance.

In this application note, this approach is described and validated using model compounds with well-characterized human pharmacokinetics and subsequently applied in case studies of aripiprazole and piroxicam. Together, these examples demonstrate how formulation-dependent dissolution and permeation data can be translated into quantitative predictions of absorption kinetics to support formulation development and bioequivalence assessment.

Absorption Model Validation and Mechanistic *In Silico* Modeling

Pion's Predictor™ software applies a modified mechanistic model based off the Gastrointestinal Unified Theoretical (GUT) framework[1] to appropriately scale *in vitro* permeation values to levels consistent with *in vivo* passive transcellular absorption. Using the scaled data, Predictor provides biorelevant predictions of *in vivo* absorption rate, total mass of drug absorbed, and the absolute fraction of a dose absorbed versus time.

To establish confidence in the mechanistic absorption model prior to its application to drug formulations, a validation exercise was performed using a diverse set of reference compounds with well-characterized human absorption profiles. Seven drugs spanning BCS Classes I, II, and IV that exhibit a wide range of *in vivo* fraction absorbed (Fa%) values were evaluated using biorelevant dissolution-permeation data as the primary model input.

Assays for the validation set were conducted using the Pion MicroFLUX™ and BioFLUX™ systems. (Figure 1 and 2). Designed to simulate intestinal absorption, these systems include donor and acceptor compartments separated by a lipid-coated artificial membrane. Drug concentrations in both the donor and acceptor compartments

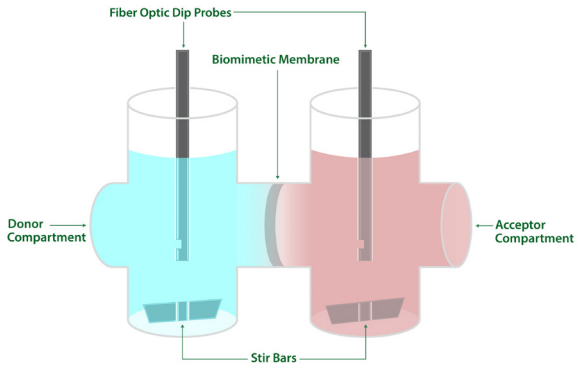
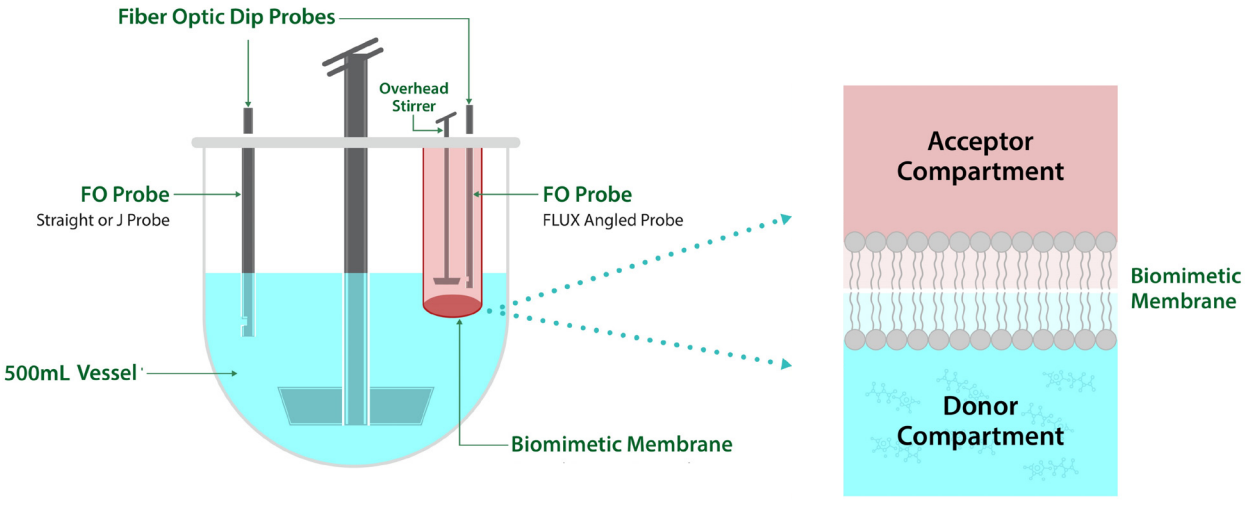


Figure 1. Schematic of the MicroFLUX dissolution–permeation apparatus.

were quantified using a Rainbow R6™ UV-vis spectrometer to assess real-time drug dissolution and permeation.

For the validation experiments, 20 mL of V1 fasted-state simulated intestinal fluid (FaSSIF) was used as the donor medium in the MicroFLUX, with 20 mL of acceptor sink buffer in the acceptor chamber.

For the BioFLUX, 250 mL of V1 FaSSIF was used as the donor medium, with 15–20 mL of acceptor sink buffer in the acceptor chamber. For selected compounds (aripiprazole, telmisartan, albendazole,



and sitagliptin), an *in situ* media change was employed to simulate gastric-to-intestinal transit, converting simulated gastric fluid (pH 1.6) to pH 6.5 V1 FaSSIF at 30 minutes via addition of FaSSIF concentrate.

Dissolution-permeation profiles generated using MicroFLUX and BioFLUX were imported into Predictor software, where donor volumes were scaled to intestinal volumes to calculate clinically relevant dose values for all compounds. Predictor's *in silico* absorption model was then used to convert permeation data into *in vivo* absorption rate and Fa% values based on physiological factors and compound-specific properties. The predicted Fa% values were subsequently compared with clinical *in vivo* Fa% values reported in the literature and on product labels.

As shown in Figure 3, a strong linear relationship was observed between predicted and reported *in vivo* Fa% values across the validation set, with a slope close to unity and an R^2 of 0.98, indicating minimal systematic bias and high predictive accuracy.

This validation exercise demonstrates that the integrated dissolution-permeation and *in silico* modeling approach can reliably translate *in vitro* dissolution-permeation measurements into meaningful *in vivo* absorption predictions across multiple BCS classes. With this foundation established, the framework was applied to evaluate formulation-dependent absorption behavior in two case studies.

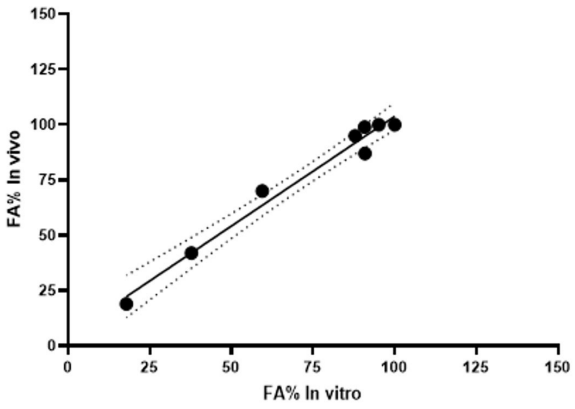


Figure 3. Plot of in vivo Fa% values versus fraction absorbed values calculated using the in silico absorption model. Slope (95% CI) = 0.99 (0.84 to 1.15), R2 = 0.98. n=8.

Reported *In Vivo* Fa% and Predicted Fa% Values

Drugs (Formulation)	*In Vivo* Dose (mg)	Predicted Fa%	Expected *In Vivo* Fa%	Fa% Reference
Aripiprazole (Abilify)	15	90.9	88[1]	(Product Label, 2014)
Albendazole (Drug)	200	17.83	19	(Sugano, 2012, p. 295)
Carbamazepine (Tegretol)	200	95.13	100	(Sugano, 2012, p. 295)
Carbamazepine (Tegretol)	400	59.45	70	(Sugano, 2012, p. 295)
Sitagliptin (Januvia)	100	87.86	95	(Sugano, 2012, p. 277)
Telmisartan (Micardis)	40	37.71	46[1]	(Product Label, 1998)
Naproxen Sodium (Aleve)	220	90.8	99	(Sugano, 2012, p. 274)

Table 1. Reported in vivo Fa% and predicted Fa% values of validation drugs calculated from the in silico modeling of dissolution-permeation data. Reference: Sugano[1]

Aripiprazole Case Study: Leveraging Dissolution–Permeation Data for Bioequivalence Prediction

In the first case study, marketed generic formulations of aripiprazole were compared to the innovator product, Abilify. Inclusion of the Abilify formulation of aripiprazole provided a direct internal benchmark of the generic formulations to a product used in the validation study.

Aripiprazole is a poorly soluble BCS Class II drug available in multiple marketed formulations. Five commercially available products were evaluated using the MicroFLUX to determine whether *in vitro* data from dose-scaled experiments could be used to accurately predict human absorption. Strong agreement with clinical data was observed across formulations, including experiments performed using whole tablets, highlighting the system's ability to maintain physiologically relevant dissolution and permeation conditions.

Fa% values were calculated from dissolution-permeation profiles using Predictor software and compared with *in vivo* Fa% values derived from clinical data. Predictions generated from both full-dose and scaled-dose experiments showed good correlation with reported absorption, with values within ±15% of the *in vivo* Fa% considered accurate (Table 2).

The aripiprazole case study demonstrates how MicroFLUX dissolution-permeation testing, combined with Predictor *in silico* modeling, can accurately estimate *in vivo* Fa% from *in vitro* data, providing formulation scientists with clinically relevant insight into oral absorption potential.

Piroxicam Case Study: Predicting *In Vivo* Performance of Nanocrystals Using *In Vitro* FLUX Measurements and *In Silico* Modeling

For the second case study, novel formulations of piroxicam (a compound not used in the validation set) were compared to the marketed brand tablet of piroxicam (Felden®).

Nanoforming technologies such as Controlled Expansion of Supercritical Solution (CESS®) offer a solvent-free approach to producing drug nanocrystals with precisely controlled particle size and increased surface area. While such systems often demonstrate enhanced dissolution *in vitro*, translation to improved *in vivo* performance is not guaranteed, particularly for nanocrystalline formulations where interfacial phenomena and dynamic dissolution-precipitation behavior can influence pharmacokinetics.

To further evaluate the predictive capability of the integrated *in vitro-in silico* framework, piroxicam, a poorly soluble compound with well-documented, formulation-dependent absorption, was selected as a model drug. The study outlined below examined whether dissolution-permeation data integrated into Predictor's mechanistic absorption model could accurately capture formulation-specific pharmacokinetic behavior across dosage forms.[2]

Dissolution-permeation studies were performed using the BioFLUX under biorelevant dosing conditions, and the resulting data were incorporated into Predictor with adjustments accounting for dissolution kinetics and particle-related diffusion effects. The resulting simulations generated plasma concentration-time profiles that were compared directly with observed *in vivo* data.

Calculated Fa% Values

Formulation	*In Vivo* Fa%	Accepted Range	Calculated Fa%	Acceptance
Abilify	87	72-102	93.9	✔
Restigulin	89	74-104	98.6	✔
Sandoz	79	64-94	91.1	✔
Piprason	92	77-107	99.6	✔
Explemed	93	78-108	87.7	✔
Abilify oral solution	87	72-102	89.8	✔

Table 2. Calculated Fa% values presented based on scaled dose measurements relative to in vivo fraction absorbed values for fasted-state conditions in humans.

Figure 4 illustrates the close agreement between the predicted and observed plasma concentration-time profiles in humans, along with corresponding pharmacokinetic parameters, including C_{max}, t_{max}, and AUC. Although total drug absorption was similar between the nanocrystalline and reference formulations, the nanocrystalline formulation exhibited a shorter t_{max}, reflecting its faster dissolution rate. Consistent with the low intrinsic membrane permeability and high ionization of piroxicam at the experimental pH, membrane diffusion remained the primary determinant of permeation and, therefore, overall absorption.

Overall, this case study demonstrates how combining dissolution-permeation testing with Predictor *in silico* modeling can resolve formulation-dependent effects on oral absorption and identify rate-limiting processes governing oral bioavailability. These findings support the utility of the approach as a predictive tool for evaluating nanocrystal formulations and guiding formulation optimization and translational decision-making.

Conclusion

Accurately predicting the *in vivo* performance of poorly soluble drugs remains a significant challenge in oral drug development, particularly for BCS Class II compounds where dissolution and permeation are closely linked. This application note demonstrates how integrating biorelevant dissolution-permeation testing with Predictor's mechanistic *in silico* absorption model provides a robust framework for addressing this challenge.

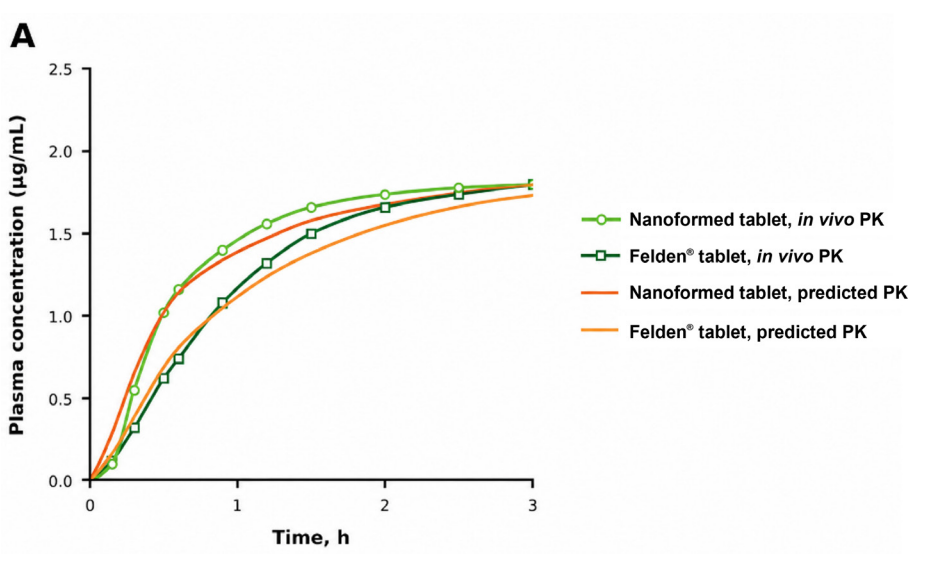


Figure 4. In vivo and in silico PK parameters for piroxicam in humans. (A) Geometric mean plasma concentration vs time profiles for 20 mg IR PRX nanocrystal tablets and reference 20 mg Felden® tablets in humans: Comparison of in silico predictions with clinical study. (B) Summary of PK parameters for each PRX form in humans: Comparison of in silico predictions with clinical study data.

***In Vivo* and *In Silico* PK Parameters for Piroxicam in Humans**

PRX Tablet and Dose	t_{max} (h)a	C_{max} (ng/mL), (CV)	$AUC_{0\text{-}1\ hr}$ (ng.h/mL), (CV)	$AUC_{0\text{-}24\ hr}$ (ng.h/mL), (CV)
Nanoformed Tablet 20 mg (*In Vivo*)	1.750 (0.75 – 4.00)	2230 (15.6)	1150 (39.3)	36200 (10.8)
Felden® Tablet 20 mg (*In Vivo*)	2.750 (0.75 – 12.00)	2230 (18.8)	863 (49.1)	38200 (13.0)
Nanoformed Tablet 20 mg (*In Vitro* + *In Silico*)	2.23 (0)	1944 (4.1)	1086 (4.5)	37678 (4.2)
Felden® Tablet 20 mg (*In Vitro* + *In Silico*)	3.52 (0)	1874 (3.9)	869 (3.9)	36570 (3.9)

Initial validation of the absorption model established strong predictive performance across multiple BCS classes, building confidence in the translation of *in vitro* dissolution-permeation data to *in vivo* Fa%. Application of this framework to aripiprazole showed that optimized dissolution-permeation testing, combined with direct use of full dissolution-permeation profiles *in silico*, can accurately predict bioequivalence outcomes under clinically relevant conditions.

Extension of the approach to piroxicam further demonstrated its ability to compare formulation-dependent influences on absorption. By capturing key pharmacokinetic parameters such as C_{max}, t_{max}, and AUC, the integrated workflow provided mechanistic insight into rate-limiting steps governing oral bioavailability and enabled meaningful comparison across formulations.

By bridging the gap between *in vitro* performance and expected *in vivo* outcomes, Pion's FLUX technology and *in silico* absorption model supports more informed formulation decisions, reduces development risk, and helps accelerate the selection and optimization of oral drug products.

References

1. Sugano K. (2012). *Biopharmaceutics Modeling and Simulations.* Wiley. https://doi.org/10.1002/9781118354339
2. Kádár S, Kennedy A, Lee S, Ruiz R, Farkas A, Tőzsér P, Csicsák D, Tóth G, Sinkó B, Borbás E. 2024. Bioequivalence prediction with small-scale biphasic dissolution and simultaneous dissolution-permeation apparatus—An aripiprazole case study, European *Journal of Pharmaceutical Sciences*, 198:106782. https://doi.org/10.1016/j.ejps.2024.106782.
3. Lee S, Shuddhodana S, Bowdery D, Arisan, A, Tsinman O, Haeggstrom E, Sinkó B, Danilova T. Predicting *In Vivo* performance of Piroxicam and Apalutamide Drug Nanocrystals from *In Vitro* Flux Measurements Combined with *In Silico* Modelling. 2026.

In Vitro + *In Silico*: Predictive Modeling

Data from BioFLUX and MicroFLUX experiments can be integrated into Pion's Predictor software to provide biorelevant absorption insights and predict *in vivo* formulation performance.

Pion Predictor

Powered by the GUT Framework

Predictor software applies the mechanisms of the Gastrointestinal Unified Theoretical (GUT) framework[1] to convert *in vitro* dissolution-permeation data to estimations of *in vivo* oral absorption, percent fraction absorbed (FA%), and the rate-limiting factor to absorption.