*p*CEL™ software

in-ADME RESEARCH
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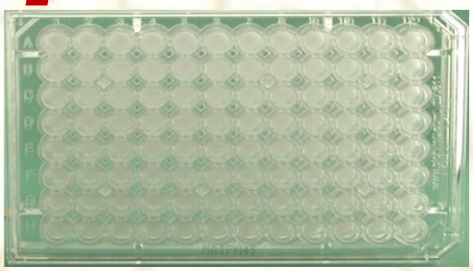


- optimize Caco-2 assay
- minimize aqueous boundary layer
- correct for paracellular diffusion
- predict Caco-2, BBB, PAMPA
- calculate $f_{u,br}$, $V_{u,br}$, $K_{p,uu}$

Interpretations of cell-based (e.g., Caco-2 or MDCK) permeability assays of lipophilic drugs can be daunting, due to the presence of the aqueous boundary layer (ABL) and paracellular diffusion. The *p*CEL-X data simulation and data refinement program can 'lift the fog, to let in the sunshine.' Better yet, assay design optimized by *p*CEL-X can minimize the ABL (or practically eliminate it). *p*CEL-X can enhance the value of Caco-2 data by extending the dynamic range of the assay, in some cases by as much as three orders of magnitude.

The Caco-2 P_{app} assay has grown in use over the past twenty-five years and is considered the standard for *in vitro* intestinal permeability assessment in the pharmaceutical industry.

Various detection methods are popular. Using LC/MS increases an already high cost of measurement, and ties down a valuable resource. Using radiolabeled compounds limits the number of molecules that can be studied. UV has been shown to work in most cases, but the UV plate reading and data analysis can be a challenge.

in-ADME Research's expertise in analyzing Caco-2, MDCK, BBB, and PAMPA assays is now available to those using Caco-2 or other cell-based assays to assess the permeability of drug candidates.

*p*CEL-X is highly trained and intelligent. It can predict the Caco-2 permeability of molecules before they are synthesized, based on an internal database of 687 curated Caco-2 published measurements.

Expertise at your fingertips

Fig1 shows plots of log P_{app} vs. lipophilicity (pH 7.4), based on a MDCK study. For lipophilic molecules, a great deal of expense was spent on measuring the easily predicted property of drug diffusion in water, *i.e.*, aqueous boundary layer (ABL) permeability, P_{ABL}. However, that says little about the transcellular permeability or the effects of efflux or other carrier-mediated transport. This conclusion can be reached by *p*CEL-X analysis of the data.[1] Better yet, the software can optimize assay design to avoid the P_{ABL} limit.[2]

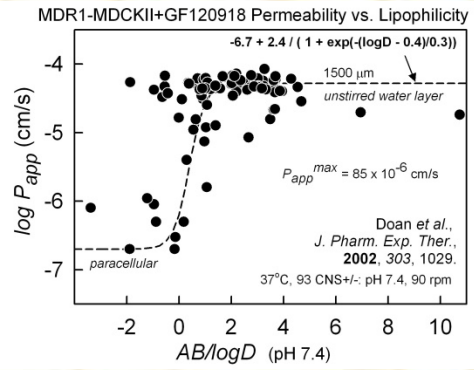


Fig 1. Plots of log P_{app} vs. calculated octanol-buffer partition coefficients (pH 7.4), where stirring was ineffective. The plots show P_{app} reaching a limiting value for log D_{OCT} > 0. About 70% of the 93 drugs were ABL-limited. (Plots from ref. 5.)

Intelligence for molecules

The way P_{app} depends on such factors as the ABL, paracellular, charged-species, or carrier-mediated transport, can be revealed from a single molecule measured at several pH values.

Neuhoff *et al.*[3,4] studied the pH-dependent transport of a series of acids and bases. Two examples are shown in Fig 2. A lot can be learned about the various factors likely to be contributing to the transport of a molecule across the cellular monolayer. If 'pure' transcellular permeability were to be measured, the optimum pH would be 6.5 for metoprolol and 7.4 for indomethacin.

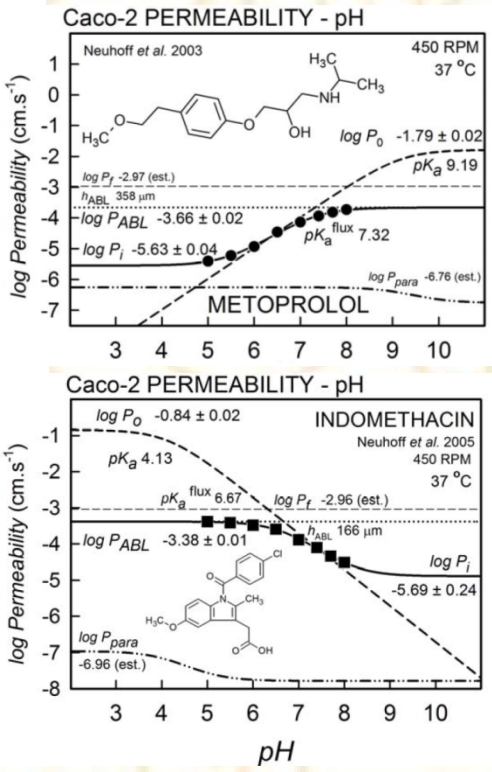


Fig 2. The Caco-2 permeability-pH of a base[3] and an acid.[4] Both assays were stirred at 450 RPM. The best-fit (*p*CEL-X) of the measured P_{app} is indicated by the solid curve. The transcellular permeability is indicated by the thick dashed curve. The ABL puts a limit on the observed permeability, as indicated by the dotted line. Neither molecule is affected by the paracellular or filter contributions (dash-dot-dot and small dashed lines). (Plots from ref. 5.)

If a measurement at a second pH were done, the ABL would be determined at pH 8 for the base and 5 for the acid. If a third pH were done, then the putative carrier-mediated effects could be addressed at pH 5 (base) and 8.5 (acid).[3,4] *p*CEL-X can predict the optimal pH for each of the above conditions before the assay is done.[2] Depending on the requirements of the study, assay may be run at 1-3 optimal pH (usually not 7.4), from which *p*CEL-X could confidently predict values at 'standard' pH 7.4, or 6.8, or any other pH). *p*CEL-X is uniquely positioned to help account for the contributions from various mechanisms of transport, an example of which is shown in Fig 3.

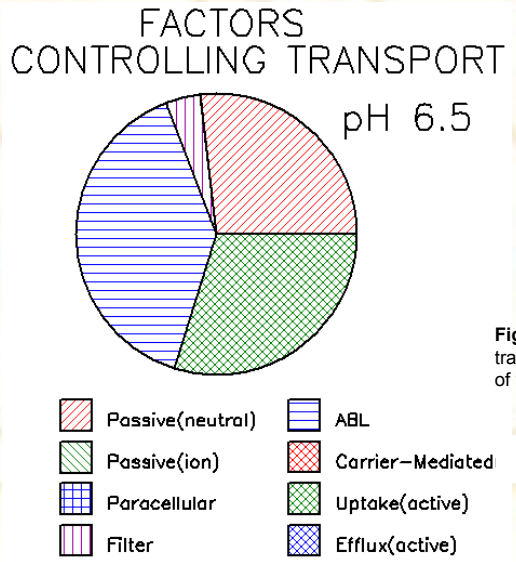
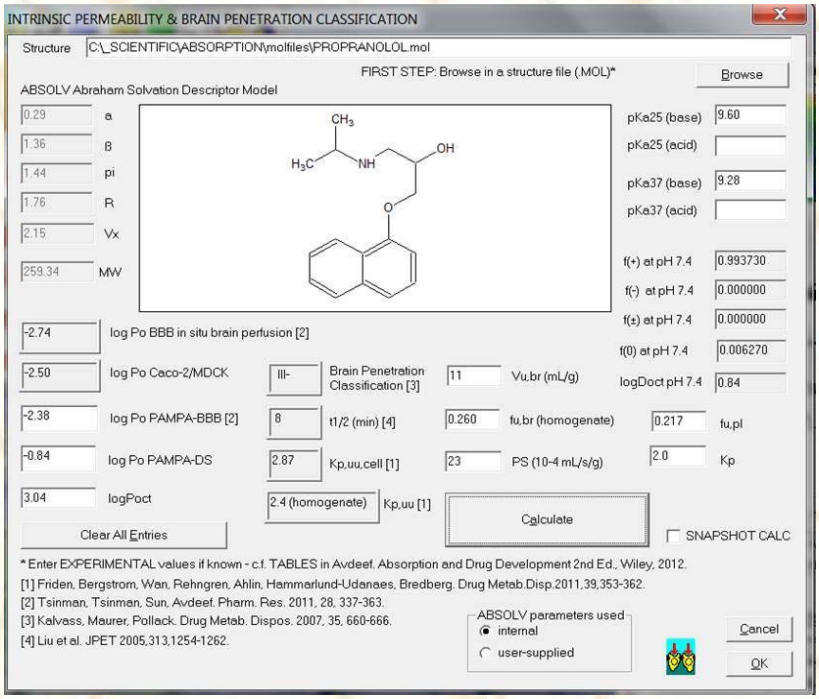


Fig 3. The factors controlling transport in a Caco-2 assay of verapamil[1] at pH 6.5.



PREDICTION of Caco-2 & PAMPA PERMEABILITY from 2-D STRUCTURE

- Internal databases of Caco-2, Double-Sink PAMPA, and PAMPA-BBB measurements are used to calculate permeability coefficients as a function of pH, using 2-D structural information (sdf format) and calculated Abraham linear free energy relations (LFER) solvation descriptors. The simulation part of *p*CEL-X is useful for scrutinizing results reported in publications, for testing new ideas concerning the mechanisms of transport, and for predicting transport properties of molecules not yet synthesized.

REFINEMENT of TRANSPORT MODELS BASED on MEASURED Caco-2, PAMPA, BMEC, or BLOOD-BRAIN BARRIER PERMEABILITY-pH DATA

- Measured permeability vs. pH data [blood-brain barrier (BBB), Caco-2/MDCK, brain microvascular endothelial cell (BMEC) or PAMPA] can be processed by *p*CEL-X to determine mechanisms of transport possibly indicated by the test compounds. Weighted nonlinear regression analysis is at the core of the program. In the case of cellular models, methods to separate carrier-mediated from passive diffusion components of transport have been tested.[1]

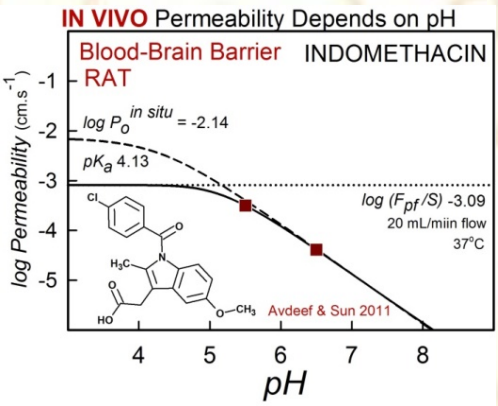


Fig 4. Example of *p*CEL-X analysis of blood-brain barrier permeability as a function of pH in rat *in situ* brain perfusion.

References with *p*CEL-X Application

[1] Avdeef A, Artursson P, Neuhoff S, Lazarova L, Gräsjö J, Tavelin S. Caco-2 permeability of weakly basic drugs predicted with the Double-Sink PAMPA pK_a^{flux} method. *Eur. J. Pharm. Sci.* **2005**, *24*, 333-349.

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[4] Neuhoff S, Ungell A-L, Zamora I, Artursson P. pH-dependent passive and active transport of acidic drugs across Caco-2 cell monolayers. *Eur. J. Pharm. Sci.* **2005**, *25*, 211-220.

[5] Avdeef A. *Absorption and Drug Development Second Edition*. Wiley-Interscience, Hoboken, NJ, 2012.

[6] Yusof SR, Avdeef A, Abbott NJ. In vitro porcine blood-brain barrier model for permeability studies : *p*CEL-X software pK_a^{FLUX} method for aqueous boundary layer correction and detailed data analysis. *Eur. J. Pharm. Sci.* **2014**, *65*, 98-111.

[7] Smith D, Artursson P, Avdeef A, Di L, Ecker GF, Faller B, Houston JB, Kansy M, Kerns EH, Krämer SD, Lennernäs H, van de Waterbeemd H, Sugano K, Testa B. Passive lipoidal diffusion and carrier-mediated cell uptake are both important mechanisms of membrane permeation in drug disposition. *Mol. Pharmaceutics* **2014**, *11*, 1727−1738.

Please note: publication-quality customized plots shown here were drawn using SigmaPlot ® (Systat Software, Inc.), based on *p*CEL-X calculation results exported to Microsoft® Excel.

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