The aim of the study was to predict the pH-dependent permeability profiles of drugs in a Caco-2 assay, using an in combo procedure based on measured Double-Sink™ PAMPA permeability values and the calculated Abraham solvation descriptors (a, b, n, R, V_j).

**ABSTRACT**

Cellular (e.g., Caco-2) and low-cost parallel artificial membrane permeability assays (PAMPA) are used to predict intestinal absorption properties of compounds in drug discovery projects. However, prior to doing these assays, reliable in silico prediction of the permeability of test compounds can be very useful. For example, knowledge of the predicted property can help the medicinal chemist to design additional structural features into the molecule to improve the prospects of bioavailability. The predicted effects of intestinal pH could thus be similarly useful (Fig. 1). Furthermore, the predicted permeability value can improve assay design for poorly soluble compounds. Unwittingly, investigators sometimes report “zero” permeability for compounds that are likely to be highly permeable, because of limitations due to extremely low solubility. In such instances, a “contrary” in silico permeability alerts the chemist to critically examine the assay design.

The first successful quantitative in silico procedure to establish a correlation between PAMPA and Caco-2 was based on fluoroquinolones from three congeneric series. Several areas of inconsistencies in data treatment in cellular studies were critically assessed, to further improve the effectiveness of the in silico methods.1,5

Out of these studies, a computer program, pCEL-X (pION), was developed, which can be used to model the various factors controlling transport of drug-like molecules across artificial or cell-based membranes in permeability measurements, such as: (a) passive (uncharged), (b) passive (ionic), (c) paracellular (Renkin sieving function; electrostatic potential model), (d) aqueous boundary layer (ABL), and (e) carrier-mediated model for acids.

**RESULTS AND DISCUSSION**

The extended training set of 53 Caco-2 measurements was correlated in the in combo model to the extent of r² = 0.98, as shown in Figure 3. For example, as a test of the procedure, the pH-dependent Caco-2 apparent permeability (in units of 10⁻⁴ cm/s) at pH 7.4 of indomethacin is 5.09 x 10⁻⁴ cm/s (Fig. 4). The predicted permeability for indomethacin (n = 53) was compared to measured data reported by Alex Avdeef (Fig. 5).

**MATERIALS AND METHODS**

The method applied to correlating the Caco-2 permeability coefficients of 18 weak-base drugs to Double-Sink™ PAMPA permeability data augmented by calculated Abraham descriptors, producing r² = 0.98 (Fig. 2) was extended to a larger training set, combining 53 high-quality Caco-2 measurements (from two or more different pH) collected from the literature, and pre-processed to extract the passive permeability components (Fig. 3). Acids, bases, neutrals, and zwitterions were included in the extended training set of compounds.

The Abraham linear free energy (LFER) descriptor calculation and the computational model testing used the Algorithm Builder V1.8 and ADME Boxes V4.1 computer programs from Pharma Algorithms, Toronto (www.ap-algorithms.com). The prediction of the pH-dependent Caco-2 permeability profile took into account, transcellular passive (neutral and charged species), paracellular, aqueous boundary layer (ABL), and filter-limited permeability.

**CASE STUDY: Caco-2 PERMEABILITY OF INDOMETHACIN**

(donor pH 5 – 8, receiver pH 7.4, 450 RPM)

The simulation program can calculate Caco-2 permeability from just a 2-D structure of the molecule, provided as a mol file. The simulation first predicts the pK_a, the octanol-water partition coefficient, log P_oct, and the intrinsic (neutral species) Double-Sink PAMPA permeability, log P_{DS}^a, along with the five Abraham LFER descriptors. However, the simulation can be improved if measured pK_a, log P_{oct}, log P_{DS}^a values are provided as input (Fig. 4). In a separate diagram (Fig. 5), the aqueous boundary layer permeability is calculated, which is a function of diffusivity, stirring speed, and temperature.

Finally, the paracellular and filter permeability values are calculated (Fig. 6) from the size of the molecule, the junction pore size, the junction potential gradient, and the porosity of the supporting filter.

**REFERENCES**