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Advances in the Prediction of Gastrointestinal Absorption: Quantitative Structure-Activity Relationship (QSAR) modelling of PAMPA Permeability

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Highlights

- QSARs of PAMPA permeability are useful to predict a chemical's passive transport.
- QSAR models allow a deeper insight into the mechanisms of the membrane transport.
- Hydrophobicity and hydrogen bonding are important for membrane permeability.
- Linear or bilinear relationships exist between PAMPA permeability and log P.
- For passive transport predicted permeability is related to Caco-2 and *in vivo* data.

Abstract

Gastrointestinal absorption (GI absorption) is a key absorption, distribution, metabolism, and excretion (ADME) property when the biological effects of substances are evaluated. The Parallel Artificial Membrane Permeability Assay (PAMPA) has emerged as a primary screen for determining passive transcellular permeability, the dominant GI absorption mechanism for many drugs, thus helping with the prioritisation of the most promising lead compounds for pharmacokinetic studies. Recently the PAMPA assay has attracted increasing interest from various other industrial sectors, including cosmetics, where such non-animal models may provide a crucial source of information for *in vitro* - *in vivo* extrapolation. This method is also a reliable source of experimental data for Quantitative Structure-Activity Relationship (QSAR) modelling of GI absorption. In this investigation, published QSAR models for PAMPA were reviewed with the aim to summarise and assess critically the current state of the art. The review indicates a relatively small number of QSARs compared to some endpoints, but much consistency within the models. PAMPA permeability increases with hydrophobicity and decreases with the surface area occupied by hydrogen bond acceptor/donor atoms. The models can be applied to screening for bioactive

compounds with the potential to pass the gastrointestinal barrier as well as to design new structures with increased PAMPA permeability, thus with better expectations towards improved *in vivo* GI absorption.

Keywords: PAMPA, QSAR, gastrointestinal absorption, physico-chemical properties, model, *in silico*

Abbreviations

AUC-ROC - area under the curve of receiver operating characteristic; ADME – absorption, distribution, metabolism, and excretion; $ClogP_{oct}$ - calculated log P_{oct} values from the MacLogP software; DS-PAMPA – Double-Sink PAMPA; DOPC – dioleoylphosphatidylcholine; F – the ratio between regression and residual variances; GI absorption – gastrointestinal absorption; $HBSA_{DA}$ – hydrogen bonding descriptor developed via summing two Codessa Pro partial surface area descriptors: H-acceptors and H-donors polar surface area; HDM – hexadecane membrane; HDCA-2/TMSA – the area-weighted surface charge of hydrogen bonding donor atoms divided by the total molecular surface area; LR – linear regression; log P_{oct} – the logarithm of the octanol-water partition coefficient; log D – the logarithm of the apparent octanol-water distribution coefficient (at a particular pH); Log P^{DOPC}_0 – intrinsic permeability coefficient measured by DOPC-PAMPA; Log P^{DS}_0 – intrinsic permeability coefficient measured by DS-PAMPA; log P^{HDM}_0 – intrinsic permeability coefficient measured by HDM-PAMPA; MLR – multiple linear regression; MSE – mean squared error; MW – molecular weight; NLR – non-linear regression; P_0 – intrinsic permeability; P_a – apparent permeability coefficient measured by PAMPA assay; PAMPA – parallel artificial membrane permeability assay; P_e – effective permeability coefficient measured by PAMPA assay; PLS – partial least squares; P_{pass} – passive permeability across parallel artificial membranes; PSA – polar surface area; q – cross-validation parameter; QSAR –

quantitative structure–activity relationship; r – correlation coefficient; r^2_{cv} – cross-validated correlation coefficient; $RMSE_{cv}$ – root mean squared error of cross-validation; s – standard deviation; SA_{HA} – surface area occupied by the hydrogen-bond acceptor atoms; SA_{HD} – surface area occupied by the hydrogen-bond donor atoms; SASA – solvent accessible surface area; SVC – support vector classification; SVR – support vector regression, TPSA – topological polar surface area; TSA – total surface area; UWL – unstirred water layer.

1. Introduction

The molecular properties defining absorption, distribution, metabolism, and excretion (ADME) are crucial in drug design and in risk assessment of chemicals. In the past, up to 40% of drug candidates failed during late stage development due to poor ADME characteristics [1,2]. Introduction of robust preclinical ADME studies led to a reduction of these failures [3].

Intestinal absorption, one of the most important ADME properties, is a complex process determined by certain physiological conditions (local pH, absorptive surface area), activities of enzymes/transporters/carriers in the gastrointestinal tract and the chemical properties (solubility, molecular size and stability) of a molecule [4]. Following oral administration a molecule must pass through intestinal cell membranes by passive diffusion, carrier-mediated uptake or active transport processes before reaching the systemic circulation [5]. In addition, paracellular diffusion is considered to be the primarily transport mechanism for very small and polar molecules.

Membrane permeability is known to be a key property in the drug design pipeline: a drug intended for an intracellular target, but with poor membrane permeability, will have low efficacy. Thus, various methods to determine membrane permeability are used routinely. Since the early 1990s, cultured cell models such as human colon adenocarcinoma Caco-2 have been used to model the permeability of drugs across the intestinal wall and have become a standard method in the pharmaceutical, and other, industries [6]. More recently, the Parallel Artificial Membrane Permeability Assay (PAMPA) has started to emerge as a primary screen for passive transcellular permeability, with this route being the dominant transport mechanism in the gastrointestinal absorption (GI absorption) for 80%-95% of the commercially available drugs [7]. The assay is attractive due to its simplicity, high throughput and low cost, making it possible to test a large number of compounds in a short period of time. Further PAMPA has proven to have good day-today reproducibility and low variability, as well as comparable prediction accuracy to the Caco-2 assay when predicting *in vivo* permeation by passive diffusion [8]

In addition to its application in drug design, the PAMPA assay has attracted increasing interest from various other industrial sectors (i.e., cosmetics, industrial chemicals, biocides and plant protection products) and regulatory authorities where non-animal models may be crucial to provide a source of information for *in vitro to in vivo* extrapolation and internal exposure [9]. It is particularly important for cosmetics industry, taking into account that oral absorption is one of the elements for the safety assessments of cosmetic ingredients, particularly to interpret the relevance of oral dosing to dermal exposure. Moreover reliable *in vitro* methods to assist safety assessment are needed since the European Union has been at the forefront of the global move to ban the animal testing of cosmetic ingredients [10,11].

In addition to the attractive methodology, PAMPA is a promising source of reliable experimental data for the computational (*in silico*) evaluation of a drug's GI absorption [12]. Such models, typically Quantitative Structure–Activity Relationships (QSARs), attempt to relate PAMPA permeability to physico-chemical properties and structural descriptors. QSARs thus allow for a prediction of PAMPA permeability to be made directly from chemical structure. In addition, the models developed are useful for screening purposes and also provide an insight into the structural characteristics important for better absorption thus guiding the design of new bioactive compounds with desirable ADME properties. Whilst many models exist, there is no overall consensus over the optimal modelling approach and which conclusions may be drawn [13].

In this paper, we provide an overview of published QSAR models for PAMPA. The aim of this investigation was to summarise and review critically the relatively new field of QSAR modelling of PAMPA. It is recognised that is still a relatively new field with a limited number of models available, however, there is considerable potential for further development and application. The particular focus of this review was the fact that PAMPA permeability has been used successfully to predict the GI absorption of orally administered drugs and the use of QSAR models accelerates this determination. The models can be applied to screen bioactive compounds for their potential to pass the gastrointestinal barrier as well as for the design of new structures with the expectation of improved *in vivo* GI absorption. Further, the PAMPA based *in silico* models may provide valuable quantitative information that is highly useful and applicable in the context of risk assessment [9].

2. The PAMPA assay

PAMPA is an experimental model introduced by Kansy et al. [14] to predict the oral absorption of new therapeutic agents in a simple, reproducible and high throughput manner. The assay measures effective/apparent permeability and/or the fraction of the permeated test compound. A limitation of PAMPA is that active and efflux transporters are not modelled by the PAMPA membrane. Despite this, it has been shown that PAMPA permeability correlates well with Caco-2 cell permeability and human intestinal absorption *in vivo*, this undoubtedly being a result of the fact that most of the known drugs are absorbed via passive diffusion. The correlations were confirmed to be statistically reliable in studies utilising linear regression reported by Ano et al. [15], Fujikawa et al. [5,16], Verma et al. [17].

The common experimental set-up of the PAMPA (Fig. 1) consists of:

(1) donor and acceptor compartments, containing an aqueous solution of the test molecule and aqueous buffer initially free of the test molecule respectively;

(2) an artificial membrane, which is composed of a variety of organic solvents or phospholipid mixtures, and used to separate the donor and acceptor compartments; and

(3) a filter, used for immobilisation and stabilisation of the membrane.

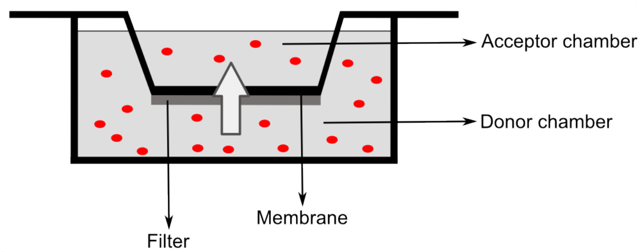

Fig. 1. A schematic diagram of the PAMPA experimental apparatus

A number of PAMPA variants have been developed which differ in:

(1) membrane composition;

(2) presence of specific ingredients in the acceptor chamber; and

(3) permeation models used for calculation of the permeability coefficients.

The most commonly used variants for modelling of intestinal absorption are: HDM-PAMPA (n-hexadecane membrane, [18]); egg-PAMPA (egg lecithin, [19]); DOPC-PAMPA (dioleoylphosphatidylcholine in dodecane membrane, [20]); BM-PAMPA (or BAMPA, biomimetic lipid mixture membrane, [21]), and DS-PAMPA (Double-Sink, lipid mixture membrane, [7]).

According to the relationship between PAMPA permeability and GI absorption reported by Avdeef [7], the value of P_e ca. $1\times10^{-6} \div 2\times10^{-6}$ may be considered as a threshold, although arbitrary, above which the compounds are classified as highly permeable in the gastrointestinal tract whereas those with P_e values lower than these are classified as being of low permeability.

3. *In silico* modelling of PAMPA permeability

The simplicity of PAMPA assay as an estimator of intestinal absorption is advantageous for development of computational models for membrane permeability prediction. Various QSAR models have been developed over the past two decades based on PAMPA permeability. The resulting models are particularly useful for initial screening of drugs, where they serve as an effective tool to guide structural modifications of the parent molecules necessary to improve permeability through biological barriers.

In addition to the QSAR PAMPA models, a number of physico-chemical / physics based models have been proposed to predict passive membrane permeation. Although better reflecting the underlying physical permeation process, they are quite expensive computationally, partly due to the complexity of the permeation process [22,23].

3.1. Main experimental properties estimated by the PAMPA assay which have been used as inputs into modelling studies

The main permeability parameters estimated from the PAMPA assay which have been used in modelling studies are the apparent and effective permeability coefficients, denoted as P_a/P_{app}, P_e, P_0 respectively. The permeability coefficient of the permeating molecule may be defined as a unit number of these molecules (mol) diffusing through a unit cross-section of the membrane (cm^2) in a unit of time (s) under a unit of concentration gradient ($mol \cdot cm^{-3}$). Consequently, it has the dimensionality of $cm \cdot s^{-1}$.

These parameters are derived from first principles and differ in some factors that influence the permeability [7]. The majority of models are developed using the apparent membrane permeability P_a as shown in Eq. 1:

$$P_a = -\frac{2.303V_D}{A \cdot t \cdot \varepsilon_a} \cdot \left(\frac{1}{1+r_v}\right) \cdot \lg\left[1 - \left(1 + \frac{1}{r_v}\right) \cdot \frac{C_R(t)}{C_D(0)}\right] \quad \text{(Eq. 1)}$$

Where:

$r_v = V_D/V_R$, V_D is the donor compartment volume of a buffer solution and V_R is the receiver compartment volume of a buffer solution;

C_R and C_D are the concentrations of the studied compound in the receiver and donor compartments, respectively, determined in the initial moment t = 0 and after a certain permeation time t;

A is the area of the membrane and ε_a is the apparent filter porosity.

It should be kept in mind that apparent permeability does not consider membrane retention and steady state time values. The parameter that takes into account these properties is the effective membrane permeability (P_e) thus providing a more reliable estimate in comparison to the apparent permeability. The P_e is defined as follows:

$$\begin{aligned} P_e &= -\frac{2.303V_D}{A \cdot (t - \tau_{SS}) \cdot \varepsilon_a} \cdot \left(\frac{1}{1+r_V}\right) \cdot \lg\left[-r_V + \left(\frac{1+r_V}{1-R_M}\right) \cdot \frac{C_D(t)}{C_D(0)}\right] = \\ &= -\frac{2.303V_D}{A \cdot (t - \tau_{SS}) \cdot \varepsilon_a} \cdot \left(\frac{1}{1+r_V}\right) \cdot \lg\left[1 - \left(\frac{1+r_v^{-1}}{1-R_M}\right) \cdot \frac{C_R(t)}{C_D(0)}\right] \end{aligned} \quad \text{(Eq. 2)}$$

Eqs. 1 and 2 are nearly identical with the only differences related to the $(1 - R_M)$ term (to reflect membrane retention − mole fraction of compound retained by the membrane R_M) and the lag time offset τ_{ss} (the time needed to saturate the membrane with solute before reaching steady state conditions).

In addition to the above discussed permeability coefficients, intrinsic permeability P_0 has been reported in some studies. It is determined for the uncharged form of the compound, and is therefore the maximal P_e for a range of pH values

Historically, instead of the permeability coefficients, the percentage flux (%F) or percentage transported solute (%T) were used to measure the fraction of the test compound which passed into the acceptor compartment over the time of experiment. Whilst being the most basic measured values in any PAMPA setup used for calculation of the permeability coefficients, %F and %T do not account for many factors in Eqs. 1 and 2, e.g.the time course of the permeation process, the membrane retention, etc.

3.2. Main datasets of PAMPA permeability for modelling purposes

A number of datasets with PAMPA permeability values published in the literature are summarised in Table 1, where the permeability parameter estimated, literature sources and the main experimental conditions are presented. The selected datasets in Table 1 are those with higher numbers of compounds (at least 40) in order to provide a solid basis for derivation of statistically reliable models. For instance, in order to avoid chance correlations at least five to six data points per variable (structural descriptors or physico-chemical properties) are required [24]. The largest publically available dataset has been collected by Avdeef and coworkers and summarised in [7]. It comprises Double-Sink PAMPA intrinsic and effective permeability coefficients determined for nearly 300 compounds (mostly commercial drugs) and was noted a benchmark dataset for PAMPA by Przybylak et al. [12]. Most of the larger datasets consist of commercial drugs as well and are constructed to evaluate PAMPA assay modifications.

It should be kept in mind that the experimental conditions used for PAMPA vary e.g. lipid composition, donor and acceptor buffer solutions, stirring speed, etc. These variations may significantly affect permeability as well as *in vivo* predictability, thus careful selection of experimental conditions is needed particularly when the modelling relies on literature data. In addition to the above mentioned conditions, pH may also strongly affect PAMPA permeability. The considerable pH gradient during the gastrointestinal transition suggests a range of pH measurements should be performed and used for *in vivo* prediction [8].The largest dataset, noted in the literature (ca. 5500 compounds) is reported by Sun et al [25] but was not considered further as it is not publicly available.

Table 1. The main datasets of PAMPA permeability existing in the literature (containing data for more than 40 compounds and excluding skin-PAMPA and BBB-PAMPA datasets).

Source	**Estimatedparameter**	**Number of substances**	**PAMPA experimental conditions**
Kansy et al. [26]	%F	94	pH 7.4, with and without 0.5% glycocolic acid
Sugano et al. [21]	P_a	80	3% BAMPA phospholipid mixture in 1,7-octadiene, pH 6
Zhu et al. [27]	P_a	92	1% egg lecithin in *n*-dodecane, pH 5.5 and 7.4
Kerns et al. [28]	P_e	72	phosphatidyl choline in dodecane, pH 4, 6.6, 7.4 and 8.0
Fujikawa et al. [16]	P_a	97	10% lecithin in 1, 9-decadiene, pH 7.3
Galinis-Luciani et al. [29]	P_e	40	20% lecithin in decane pH 7.4
Chen et al. [30]	P_e	47	lipid/oil/lipid tri-layer artificial membrane (phospholipids mixture-hexadecane- phospholipids mixture), pH 7.4
Avdeef [7]	P_0, P_e	292	Double-Sink PAMPA, pH 6.5 and 7.4, UWL-adjusted setup
Wang et al. [31]	P_a	62	1,2-dioleoyl-*sn*-glycero-3-phosphocholine in hexadecane
Oja and Maran [32]	P_e	80	1% lecithin in dodecane , pH 3, 5, 7.4 and 9
Oja and Maran [33]	P_e	97	1% lecithin in dodecane , pH 3, 5, 7.4 and 9
Oja and Maran [34]	P_e	75	1% lecithin in dodecane , pH 3, 5, 7.4 and 9

Source	Estimatedparameter	Number of substances	PAMPA experimental conditions
Oja and Maran [35]	P_e, P_0	238	1% lecithin in dodecane , pH 3, 5, 7.4 and 9

3.3. QSAR models of PAMPA permeability

The usability of QSAR models for PAMPA relies on the fact that permeability (particularly when combined with aqueous solubility and pKa) can be applied as a predictor of GI absorption of orally administered drugs [7,36].

The models reported in the literature follow the classical QSAR approach employing experimental and/or theoretical structural descriptors to formulate a relationship with permeability. Most models are based on MLR or PLS statistical methods. Only a small number of models apply artificial neural networks to incorporate nonlinear dependencies. Generally, simple models based on few descriptors are easier to interpret than models based on many descriptors relating permeability in a complex way [37]. In addition the main requirements for good QSAR practice have to be followed, including thorough model validation and applicability domain analysis [38].

QSAR models for PAMPA relate the relevant permeability parameter, e.g. P_a, K_p or flux, with physico-chemical properties such as the logarithm of the octanol-water partition coefficient (log P), the negative logarithm of the acid dissociation constant (pKa) and the logarithm of the octanol-water distribution coefficient (log D) or structural descriptors including the polar surface area (PSA), the surface area of hydrogen bond donors or acceptors; Abraham solute descriptors; indicator variables for specific functional groups; VolSurf parameters, etc (Table 2).

In related studies QSAR models for PAMPA data of peptide derivatives and other structures were developed [5,15,16] using classical structural properties (log P, pK_a and hydrogen-bonding potential) and VolSurf parameters. The models were based on Multiple Linear Regression (MLR) and/or Partial Least Squares (PLS) (Table 2).

Specifically, Ano et al. [15] analysed the absorption of peptide-related compounds across the membrane via a transcellular route, using the PAMPA assay. Although Caco-2 cells were extensively utilised to evaluate metabolism and absorption of compounds, it was shown that many peptide derivatives were hydrolysed by both extra- and intracellular enzymes produced by Caco-2 cells during permeation. In this case, the PAMPA assay is especially valuable as it enables the measurement and understanding of the passive diffusion properties of these compounds. Log P_a values were measured at pH 7.3 and 6.3 and predictive models were built for both experimental conditions, using classical and VolSurf [39] parameters. The results from both methods showed that the most significant physico-chemical features to determine variations in PAMPA permeability were the hydrogen bonding ability of molecules in addition to hydrophobicity at a particular pH. According to the models, an increase in apparent hydrophobicity at a particular pH increases permeation across the membrane whereas larger surface area occupied by hydrogen bond acceptor atoms is unfavourable for permeation through membranes. A plot of Caco-2 versus PAMPA permeability coefficients was derived for the compounds tested and provided a means to arrange compounds according to their absorption pathway.

Fujikawa et al. [5] measured and analysed PAMPA permeability of various drugs, in addition to the already reported peptide-related compounds. The predictive models derived by the same modelling approach revealed that the hydrogen-donating ability, in addition to hydrogen-accepting ability, is significant in determining the PAMPA permeability of compounds with more diverse structures. An equation with acceptable statistical quality was reported which employed calculated physicochemical parameters. The equation implied it may be possible to predict passive transport using calculated values. An *in silico* stepwise procedure that takes into account the contribution of both active and passive transport mechanisms was proposed for prediction of intestinal absorption based on the correlation between PAMPA and Caco-2 permeability coefficients and human intestinal absorption.

In an even more extensive study, Fujikawa et al. [16] experimentally measured the P_a of more compounds with high apparent hydrophobicity, including several pesticides. In the previous study [5] compounds such as desipramine, imipramine, and testosterone were excluded from the analyses as their measured P_a values were lower than calculated. In order to address this phenomenon, the role of the unstirred water layer (UWL) on membrane surfaces and the membrane retention was examined and the influence of these factors on PAMPA permeability of hydrophobic compounds was clarified. It was determined that the UWL in a typical unstirred PAMPA setup is about an order of magnitude thicker than that in the intestine (mainly due to the intestinal microvilli movements) and acts as a rate-limiting barrier for PAMPA permeability of hydrophobic compounds. Taking this into account, the PAMPA permeability of the whole dataset of hydrophilic and hydrophobic compounds was explained by a bilinear QSAR model, which includes the same parameters used in the previously reported equations. Since many chemicals have toxic effects, the proposed model can be used to support the safety assessment of chemicals to which humans are inadvertently exposed, in addition to screening for drug candidates.

Wang et al. [31] correlated permeability through PAMPA with experimentally measured parameters of cyclic peptides obtained by using rapid assays based on chromatography and nuclear magnetic resonance spectroscopy. A bilinear model was derived where HPLC capacity factor and amide temperature coefficients were shown to contribute to cyclic peptides PAMPA permeability.

The first modelling studies that account for pH differences in the intestine using QSAR modelling were recently performed by Maran and co-authors. Their first publication [32] considered the diversity of pH in the gastrointestinal tract that occurs depending on the diet (fasted/fed state) and motivated them to measure and analyse PAMPA permeabilities at different pH values between 3 and 9. The QSAR models developed based on drugs and drug-like compounds with various pKa values used the highest permeability value of a compound from all pHs thus provided a good starting point for estimation of maximum GI absorption. The best model included only two mechanistically relevant descriptors, logP and hydrogen bonding surface area. In the subsequent studies chemical class-specific QSAR analyses were performed and pH-permeability profile modelling is proposed by development of a series of equations for different pH. In [33]two groups of models were developed – for acidic and basic compounds respectively. It has been observed that the permeability of acidic compounds is influenced mainly by hydrogen bond donor properties, and the permeability of basic compounds is more dependent on partition properties. A further modelling study focused on neutral and amphoteric compounds [34]. The results showed that the membrane permeability of neutral compounds is influenced mainly by the hydrogen bond donor ability while logP is not a suitable descriptor. Amphoteric compounds have complex chemical constitution and require three-parameter models to predict membrane permeability, the parameters being log P, hydrogen bond properties and the shape of the molecules. Considering molecular size, the authors concluded that more compact molecules typically have higher membrane permeability. Further investigations by the same group [35] demonstrated that replacement of log P with log D considerably improves correlations with membrane permeability. Further, from a comparison with human GI absorption the authors introduced a cutoff value (log $P_e = -6.20$) to distinguish between high and low membrane permeability. Based on this cutoff value, classification predictions by the QSAR models were performed. Good discriminative ability between compounds with high and low permeability was demonstrated. In this study, in addition to the pH-permeability profile predictive models, models were also derived for the highest permeability and intrinsic permeability. All QSAR models developed within these studies have been validated on external datasets and, together with the experimental data, are available through the QsarDB repository [40] (http://dx.doi.org/10.15152/QDB.137 [32]; http://dx.doi.org/10.15152/QDB.166 [33]; http://dx.doi.org/10.15152/QDB.184 [34]; http://dx.doi.org/10.15152/QDB.203 [35]).

Several studies that use calculated descriptors only, as opposed to measured properties, were reported as many commercially available programs can calculate the common physico-chemical properties that have been shown to be useful for the prediction of PAMPA permeability.

The study reported by Verma et al. [17] presents various QSAR models of membrane permeability measured by PAMPA and modified PAMPA assays. Linear and bilinear relationships were obtained, in all of which the hydrophobicity of the compounds represented by ClogP plays a significant role. The bilinear dependence between hydrophobicity and PAMPA permeability is confirmed and indicator variables that reflect the importance of functional groups with hydrogen bonding capacity were identified.

The analysis of Fischer et al. [41] was focused on the permeability of charged molecules, in this case quaternary amines. Contrary to most theories, their results indicated that the permeability of permanently charged compounds can cover a wide range of values and they hypothesised that the distribution of the charge over aromatic ring systems may play a crucial role in the variations in permeability. Computational models were built for the PAMPA permeability of 20 compounds using calculated descriptors that reflect hydrophobic, H-bonding, electronic properties and shape of the molecules.

With regard to the need for effective methods for the selection of compounds with desired permeability from compound libraries, Nakao et al. [42] developed a number of PAMPA models using only *in silico* descriptors for the estimation of the permeability of compounds before they are synthesised. They proposed an *in silico* permeability prediction system, which is based on computed logP, pKa (in the form of |pKa – pH|) and PSA to predict the permeability of chemically diverse structures. A number of software programs were employed to calculate these descriptors and the best predictions were obtained with ClogP, ACD/pKa, and TPSA (calculated with Biobyte, ACD/Labs and Molinspiration software, respectively).

An MLR model with descriptors similar to those suggested by Kansy et al. [26], namely log D and the ratio of polar to total molecular surface area (PSA/TSA), was developed recently by the authors of this review [43]. Substitution of the PSA/TSA ratio was considered to facilitate calculations: PSA was substituted by topological polar surface area (TPSA) [44], TSA was substituted by molecular weight, the most fundamental descriptor of the molecular size. For the purposes of modelling, PAMPA permeability coefficients measured by the Double-Sink, UWL-adjusted PAMPA assay for 276 compounds from the dataset reported by Avdeef [7] were used. The sink conditions (lowering the active concentration of free permeant in the acceptor compartment) together with the UWL control (achieved by in-well stirring) allowed for elimination of non-linearity of the P_e data across a broad range of lipophilicity. The models derived proved to have high predictivity (external validation $q^2 = 0.77 \div 0.79$) and were implemented in the open source knowledge-mining platform KNIME [45] where can be easily applied for screening of chemical libraries to select compounds with suitable permeability [46].

More QSAR models with calculated descriptors were reported by Karelson et al. [47] and Tulp et al. [48]. Charge distribution, polarisability, hydrogen acceptor/donor potentials, molecular shape and surface area were considered as descriptors in the equations. The models from both analyses [47,48] exhibit reasonable prediction capabilities.

Sun et al. [25] reported a support vector regression (SVR) model with high predictive ability based on a dataset obtained from the National Center for Advancing Translational Sciences (NCATS). The dataset included 4071 compounds with quantitative permeability data which acted as the training set and 1364 compounds with qualitative permeability data which acted as the test set. The predictive ability of the model was estimated by the area under the curve of receiver operating characteristic (AUC-ROC) parameter. The AUC-ROC value of 0.90 indicated high predictivity of the SVR PAMPA model. The key features influencing the permeability of a compound identified by the model were PSA, counts of HBD and HBA, MW and the occurrence of an acidic group in the molecule. On line PAMPA permeability predictor based on the developed models is available (https://tripod.nih.gov/adme/pampar/ppp.html).

A small number of QSPR models relate PAMPA permeability parameters to the Abraham linear free energy solvation descriptors [49] in order to shed light on important relationships between permeability values measured by different PAMPA assays.

Ruell et al. [50] reported a UV-detection PAMPA cosolvent procedure based on the use of 20% v/v acetonitrile in the aqueous buffer. Cosolvents [21] as well as bile salts [26] and other solubilising agents [51] are known to improve the permeability assay of poorly soluble compounds, which is especially valuable to measure the intrinsic permeability of weakly soluble molecules. A training set of 32 drugs was studied both in aqueous buffer and in cosolvent-buffer solution and the intrinsic permeability coefficients were determined by the pK_a^{flux} method. A unified acid-base *in silico* permeability model was derived which revealed the dependence of the intrinsic permeability coefficients on the properties of the two solvent systems. The best MLR equation included Abraham's H-bond acidity (α) and basicity (β) and the intrinsic permeability value measured in cosolvent solution, P_o^{COS}. The model was used to calculate the aqueous intrinsic permeability, P_0, of five widely used drugs with low solubility, which could not be characterised without cosolvent.

The study of Avdeef and Tsinman [52] compared the intrinsic permeability coefficients (Log $P^{HDM}{}_0$, Log $P^{DOPC}{}_0$ and Log $P^{DS}{}_0$) of drug molecules, obtained by three variants of the PAMPA assay, namely HDM-, DOPC- and DS-PAMPA. It was shown that permeability parameters measured by these assay variants differ substantially, with up to 1000-fold difference between values derived from DS- and HDM-PAMPA models. Therefore, an *in combo* approach was adopted where a measured descriptor (DS-PAMPA) is "combined" with *in silico* descriptors to predict the other permeability models (HDM or DOPC) to explain the relationships between the models. Two Abraham descriptors are identified as being significant – the solute H-bond acidity (α) and the solute H-bond basicity (β) in explanation of P_0.

One advantage of QSAR modelling for PAMPA is its ability to guide structural modifications within a series of structural analogues needed for improvement of permeability. This is well illustrated in the study of Savić et al. [53] where the permeability of 13 newly synthesised β-hydroxy-β-arylalkanoic acids (HAA) was measured in PAMPA followed by QSAR modelling with the final aim to propose novel HAA structures with improved GI absorption. Despite the limitation in the training set (it being only 8 compounds), the results indicated some significant trends: the introduction of branched side chains, as well as introduction of substituents on one phenyl ring (which disturbs symmetry of the molecule) have positive impact on the permeability and thus on GI absorption.

4. Conclusions

PAMPA is a convenient assay for the estimation of intestinal absorption of drugs and related compounds. Data from the PAMPA assay have been used to develop *in silico* models for the prediction of membrane permeability. This review has demonstrated that QSAR models of PAMPA permeability may be a valuable tool, particularly in the initial steps of drug design. They may assist drug designers in the estimation of the passive intestinal permeability of drugs and other bioactive compounds thus saving time and reducing costs as by the potential elimination of *in vivo* and /or *in vitro* experiments.

Analysis of the QSAR models in this review has shown that PAMPA permeability generally increases with hydrophobicity and decreases with the surface area occupied by hydrogen bond acceptor/donor atoms. This confirmed that passive diffusion is the more probable mechanism for smaller molecules with low PSA. With regard to hydrophobicity, linear QSARs with log P are reliable when UWL adjustment is provided in the assay. Alternatively, for assays without UWL adjustment, bilinear QSARs are more appropriate for predictive purposes.

A significant number of studies investigated the correlations between permeability in PAMPA and Caco-2 cells as well as GI absorption. In general, good correlations were identified for passively transported molecules whereas molecules where active transport may be important were shown to be poorly predicted. Therefore, analysis of such relationships is useful to identify actively transported molecules. Such types of combined approaches may be synergistically effective in covering different transport mechanisms and thus useful for *in vivo* prediction [8].

The accuracy of the predictions for PAMPA from QSAR follows the usual limitations of modelling when statistical models are being developed: accuracy depends strongly on the size, quality and representativeness of the training set used to derive the model. The current trend in the QSAR modelling field is to construct larger training sets of structures that are necessary to elucidate the proper functional relationship between molecular descriptors and experimentally determined membrane permeability rates. Concerning PAMPA modelling, only a small number of large datasets are available in the public domain. More work is needed in this direction to provide access to large diverse compounds datasets that would be useful for development of reliable and highly predictable models with a broader applicability domain. In addition, experimental conditions should be carefully considered when QSAR analysis is performed. This was shown to be particularly important if heterogeneous compilations of data are used. In particular membrane permeability measurements over wider pH ranges provide more information about passive transport and thus are a good basis for modelling.

Most of the published PAMPA, as well as GI absorption data are related to commercial drugs and drug-like molecules. Most of them have high or medium permeability that limits the predictions as a good spread of values is not always obtained. Thus further work is needed in this direction to expand the chemical space and thus the applicability domain of the developed models. This is particularly important in the light of the potential applications of these models in chemical safety assessment processes relevant to different industrial sectors such as cosmetics, industrial chemicals, biocides and plant protection products.

Table 2. A summary of QSAR models for permeability measured in PAMPA assay in chronological order.

Model, Reference	**Dependent variable**	**Class(es) studied**	**Statistical method/ Software**	**Training set**	**Significant parameters**	**Statistical parameters**
Ruell at al. [50]	Log P_o (intrinsic permeability value under cosolvent-free conditions)	Ionisible drugs (17 bases, 13 acids, 2 ampholytes)	MLR	32	log P_o^{COS} (intrinsic permeability values measured in cosolvent solution); Abraham's H-bond acidity (α) and basicity (β)	$r = 0.97$, $s = 0.38$, $F = 279$, $q^2 = 0.96$
Ano et al. [15]	Log P_a at pH 7.3 and 6.3	Peptide derivatives and related compounds	MLR	22	MLR: log P_{oct}, $\lvert pK_a - pH \rvert$, SA_{HA}	pH 6.3: $s = 0.28$, $r^2 = 0.85$;
			PLS (VolSurf analysis)	22	PLS	pH 6.3: number of components = 4; $SE_{calc} = 0.24$; $r^2 = 0.89$; $SE_{pred} = 0.49$; $r^2_{pred} = 0.55$
						pH 7.3: number of components = 4; $SE_{calc} = 0.26$; $r^2 = 0.87$; $SE_{pred} = 0.43$; $r^2_{pred} = 0.65$
Fujikawa et al. [5]	Log P_a at pH 7.3	35 commercial drugs and 22 peptide-related compounds	MLR	57	Log $P_{oct,}$ $\lvert pK_a - pH \rvert$, $SA_{HA,}$ SA_{HD}	$s = 0.32$; $r^2 = 0.78$; $q^2 = 0.74$
			PLS (Volsurf program/ analysis)			Number of components = 6; $SE_{calc} = 0.39$; $r^2 = 0.69$; $SE_{pred} = 0.56$; $q^2 = 0.37$
			MLR		Calculated physicochemical parameters: ClogP_{oct}, $\lvert$calcd $pK_a - pH \rvert$	$s = 0.36$; $r^2 = 0.72$; $q^2 = 0.67$
Avdeef and Tsinman [52]	Log P^{HDM}_0	Commercial drugs and some organic acids	MLR	31	log P^{DS}_0, Abraham solvation descriptors: α (solute H-bond acidity) and β (solute H-bond basicity)	$r^2 = 0.89$; $s = 0.60$; $F = 75$; $q^2 = 0.86$

Model, Reference	Dependent variable	Class(es) studied	Statistical method/ Software	Training set	Significant parameters	Statistical parameters
	log $P^{DOPC}{}_0$			42		$r^2 = 0.84$; $s= 0.89$; $F = 64$; $q^2 = 0.81$
Verma et al. [17]	Log P_a, F		MLR, NLR	13 - 94 in different datasets	CLOGP; four indicator variables	
Fischer et al. [41]	log P_e	Permanently positive charged compounds	PLS	20	Electronic properties and shape parameters	Best model: $r^2 = 0.89$; $r^2X = 0.71$; $q^2 = 0.72$; RMSE = 0.52; number of components = 3
Fujikawa et al. [16]	Log P_a at pH 7.3	37 chemicals, 38 commercial drugs, 22 peptides and related compounds	MLR	71	MLR (model for hydrophilic compounds): log P, |pKa - pH|, SA_{HA}, SA_{HD}	$s = 0.35$; $r^2 = 0.76$; $q^2 = 0.72$
				26	MLR (model for hydrophobic compounds): logP, |pKa - pH|	$s = 0.30$; $r^2 = 0.54$; $q^2 = 0.42$
			NLR	97	NLR (combined model): log P_{app}, SA_{HA}, SA_{HD}	$s = 0.36$; $r^2 = 0.72$; $q^2 = 0.68$
Nakao et al. [42]	Log P_a at pH 7.3	Diverse compounds including peptide related compounds, drugs, and other chemicals	MLR	60	logP, pK_a, and PSA – experimentally determined or calculated	Best model: SD = 0.389; $r^2 = 0.699$; $F_{3,56} = 43.428$; $r^2_{cv} = 0.653$; $RMSE_{cv} = 0.404$
Karelson et al. [47]	Log P_a at pH 5.5 and pH 7.4	Various drugs and drug-related compounds	MLR models	47	MLR models: Descriptors related to charge, hydrogen acceptor and donor potentials, polar molecular surface area, electrophilicity and nucleophilicity of the compounds.	Log P_a at pH 5.5: $r^2 = 0.653 \div 0.709$; $r^2_{cv} = 0.539 \div 0.591$; external validation $q^2 = 0.519 \div 0.577$ $n_{ext} = 23/24$

Model, Reference	Dependent variable	Class(es) studied	Statistical method/ Software	Training set	Significant parameters	Statistical parameters
				54		Log P_a at pH 7.4: $r^2 = 0.752 \div 0.814$; $r^2_{cv} = 0.601 \div 0.694$; external validation $q^2 = 0.444 \div 0.630$ $n_{ext} = 42/43$
			ANN models	61		Log P_a at pH 5.5: $r^2_{train} = 0.820$; $r^2_{test} = 0.740$
				52		Log P_a at pH 7.4: $r^2_{train} = 0.801$; $r^2_{test} = 0.791$ $n_{ext} \leq 10$
Tulp et al. [48]	Log P_a at pH 7.3	Peptidic compounds and commercially available drugs	MLR	60	5 descriptors that account for hydrogen bonding ability, charge distribution, polarisability, and shape of molecules	$r^2 = 0.707$; $r^2_{cv} = 0.631$; F = 18.8; $s^2 = 0.143$
Wang et al. [31]	Log P_a	Cyclic peptides	NLR	26	Log k' (HPLC capacity factor) and $\Sigma\Delta\delta_{NH}/\Delta T$ values (amide temperature coefficients)	s = 0.30; $r^2 = 0.63$; $q^2 = 0.52$
Oja and Maran [32]	Log P_e at four pHs (3, 5, 7.4, 9)	Drugs and drug-like compounds	MLR	44	Log P and $HBSA_{DA}$	$r^2 = 0.8250$; F = 96.6589; $r^2_{cv} = 0.7984$; $r^2_{scr} = 0:0489$; $s^2 = 0.2124$

Model, Reference	**Dependent variable**	**Class(es) studied**	**Statistical method/ Software**	**Training set**	**Significant parameters**	**Statistical parameters**
Oja and Maran [33]	Log P_e at four pHs (3, 5, 7.4, 9)	Acidic and basic drugs and drug-like compounds	MLR	28 acidic compounds 46 basic compounds	Acidic compounds: HDCA-2/TMSA Basic compounds: log $D_{pH7.4}$ and log D_{pH9}:	Best models for acidic compounds: $r^2 > 0.8$ for pH 3 and pH 5 external validation $q^2 = 0.31/0.64$ $n_{ext} = 8$ Best models for basic compounds: $r^2 > 0.7$ for pH 7.4 and pH 9 external validation $q^2 = 0.72/0.85$ $n_{ext} = 15$
Oja and Maran [34]	Log P_e at four pHs (3, 5, 7.4, 9)	Neutral and amphoteric Drugs and drug-like compounds	MLR	12 neutral compounds 46 amphoteric compounds	Neutral compounds: HDCA-2 Amphoteric compounds: log P; HDCA	Neutral compounds models: $r^2 = 0.95 \div 0.96$ external validation $q^2 = 0.96 \div 0.99$ $n_{ext} = 3$ Amphoteric compounds: $r^2 = 0.64 \div 0.77$ external validation $q^2 = 0.67 \div 0.73$ (after 2 outliers removed) $n_{ext} = 14$
Oja and Maran [35]	Log P_e at four pHs (3, 5, 7.4, 9), logPe_highest, and logPo calculated from pH-s and pKa	238 drug substances	MLR and threshold based classification	134	Log D and additional CODESSA descriptors to capture additional important structural characteristics	Six MLR and six classification models Model for logP_e – highest: $r^2 = 0.75$; $q^2 = 0.74$; $s^2 = 0.34$; external validation $q^2 = 0.60$ $n_{ext} = 60$

Model, Reference	Dependent variable	Class(es) studied	Statistical method/ Software	Training set	Significant parameters	Statistical parameters
Sun et al. [25]	Log P_e	Mostly drug-like molecules	SVR, SVC	SVR: 4079 SVC: 2346	PSA, counts of hydrogen bond donors (HBD) and acceptors (HBA), count of aromatic rings, molecular weight, hydroxyl oxygen and hydrogen in an acidic group	Regression model: $r^2 = 0.90$; MSE – 0.07 log units. AUC-ROC = 0.90 Classification model: AUC-ROC = 0.88
Savić et al. [53]	P_a, %T	Newly synthesised β-hydroxy-β-arylalkanoic acids	MLR, PLS and ANN;	8	Dragon descriptors (TALETE slr, 2010) indicating that introduction of branched side chain and introduction of substituents on the one phenyl ring could have positive impact	Best (MLR) models MLR (%T): $r^2 = 0.990$; SEE = 0.916; F = 209.16; LOO $q^2 = 0.942$; external validation $q^2 = 0.976$. MLR (P_{app}): $r^2 = 0.852$; SEE = 0.248; F = 209.16; LOO $q^2 = 0.645$; external validation $q^2 = 0.901$ $n_{ext} = 5$
Diukendjieva et al. [43]	P_e	Drugs and drug-like compounds	MLR	251	Log D, TPSA/MW	ACD/Percepta log D model: $r^2 = 0.75$; SEE = 1.10; F = 371.3; LOO $q^2 = 0.74$; external validation $q^2 = 0.79$ (n_{train} / n_{ext} = 200 / 51)

Model, Reference	Dependent variable	Class(es) studied	Statistical method/ Software	Training set	Significant parameters	Statistical parameters
				248		ChemAxon log D model: $r^2 = 0.74$; SEE = 1.11; F = 345.1; LOO $q^2 = 0.73$; external validation $q^2 = 0.77$ (n_{train} / n_{ext} = 198 / 50)

Competing interests:

The authors declare no competing interests.

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Referenses

[1] Li AP. Screening for human ADME/Tox drug properties in drug discovery. Drug Discovery Today 2001;6:357–366.

[2] van de Waterbeemd H, Gifford E. ADMET in silico modelling: towards prediction paradise? Nature Reviews: Drug Discovery 2003;2:192–204. doi:10.1038/nrd1032.

[3] Bocci G, Carosati E, Vayer P, Arrault A, Lozano S, Cruciani G. ADME-Space: a new tool for medicinal chemists to explore ADME properties. Scientific Reports 2017;7. doi:10.1038/s41598-017-06692-0.

[4] Martinez MN, Amidon GL. A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. The Journal of Clinical Pharmacology 2002;42:620–643.

[5] Fujikawa M, Ano R, Nakao K, Shimizu R, Akamatsu M. Relationships between structure and high-throughput screening permeability of diverse drugs with artificial membranes: Application to prediction of Caco-2 cell permeability. Bioorganic & Medicinal Chemistry 2005;13:4721–32. doi:10.1016/j.bmc.2005.04.076.

[6] Hilgers AR, Conradi RA, Burton PS. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. Pharmaceutical Research 1990;7:902–10. doi:10.1023/A:1015937605100.

[7] Avdeef A. Absorption and Drug Development: Solubility, Permeability, and Charge State. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2012. doi:10.1002/9781118286067.

[8] Sugano K. 5.19 - Artificial Membrane Technologies to Assess Transfer and Permeation of Drugs in Drug Discovery. In: Taylor JB, Triggle DJ, editors. Comprehensive Medicinal Chemistry II, Oxford: Elsevier; 2007, p. 453–87. doi:10.1016/B0-08-045044-X/00136-X.

[9] Gordon S. Non-animal models of epithelial barriers (skin, intestine and lung) in research, industrial applications and regulatory toxicology. ALTEX 2015;32:327–78. doi:10.14573/altex.1510051.

[10] Obringer C, Manwaring J, Goebel C, Hewitt NJ, Rothe H. Suitability of the in vitro Caco-2 assay to predict the oral absorption of aromatic amine hair dyes. Toxicology in Vitro 2016;32:1–7. doi:10.1016/j.tiv.2015.11.007.

[11] Williams FM, Rothe H, Barrett G, Chiodini A, Whyte J, Cronin MTD, Monteiro-Riviere NA, Plautz J, Roper C, Westerhout J, Yang C, Guy RH. Assessing the safety of cosmetic chemicals: Consideration of a flux decision tree to predict dermally delivered systemic dose

for comparison with oral TTC (Threshold of Toxicological Concern). Regulatory Toxicology and Pharmacology 2016;76:174–86. doi:10.1016/j.yrtph.2016.01.005.
[12] Przybylak KR, Madden JC, Covey-Crump E, Gibson L, Barber C, Patel M, Cronin MTD. Characterisation of data resources for in silico modelling: benchmark datasets for ADME properties. Expert Opinion on Drug Metabolism & Toxicology 2018;14:169–81. doi:10.1080/17425255.2017.1316449.
[13] Patel M, Chilton ML, Sartini A, Gibson L, Barber C, Covey-Crump L, Przybylak KR, Cronin MTD, Madden JC. Assessment and reproducibility of quantitative structure–activity relationship models by the nonexpert. Journal of Chemical Information and Modeling 2018;58:673–82. doi:10.1021/acs.jcim.7b00523.
[14] Kansy M, Senner F, Gubernator K. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. Journal of Medicinal Chemistry 1998;41:1007–10. doi:10.1021/jm970530e.
[15] Ano R, Kimura Y, Shima M, Matsuno R, Ueno T, Akamatsu M. Relationships between structure and high-throughput screening permeability of peptide derivatives and related compounds with artificial membranes: application to prediction of Caco-2 cell permeability. Bioorganic & Medicinal Chemistry 2004;12:257–64. doi:10.1016/j.bmc.2003.10.002.
[16] Fujikawa M, Nakao K, Shimizu R, Akamatsu M. QSAR study on permeability of hydrophobic compounds with artificial membranes. Bioorganic & Medicinal Chemistry 2007;15:3756–67. doi:10.1016/j.bmc.2007.03.040.
[17] Verma RP, Hansch C, Selassie CD. Comparative QSAR studies on PAMPA/modified PAMPA for high throughput profiling of drug absorption potential with respect to Caco-2 cells and human intestinal absorption. Journal of Computer-Aided Molecular Design 2007;21:3–22. doi:10.1007/s10822-006-9101-z.
[18] Wohnsland F, Faller B. High-throughput permeability pH profile and high-throughput alkane/water log P with artificial membranes. Journal of Medicinal Chemistry 2001;44:923–30. doi:10.1021/jm001020e.
[19] Kansy M, Avdeef A, Fischer H. Advances in screening for membrane permeability: high-resolution PAMPA for medicinal chemists. Drug Discovery Today: Technologies 2004;1:349–55. doi:10.1016/j.ddtec.2004.11.013.
[20] Avdeef A, Strafford M, Block E, Balogh MP, Chambliss W, Khan I. Drug absorption in vitro model: filter-immobilized artificial membranes. 2. Studies of the permeability properties of lactones in Piper methysticum Forst. European Journal of Pharmaceutical Sciences 2001;14:271–80.
[21] Sugano K, Hamada H, Machida M, Ushio H, Saitoh K, Terada K. Optimized conditions of bio-mimetic artificial membrane permeation assay. Int J Pharm 2001;228:181–8.
[22] Kalyanaraman C, Jacobson MP. An atomistic model of passive membrane permeability: application to a series of FDA approved drugs. Journal of Computer-Aided Molecular Design 2007;21:675–9. doi:10.1007/s10822-007-9141-z.
[23] Leung SSF, Mijalkovic J, Borrelli K, Jacobson MP. Testing physical models of passive membrane permeation. Journal of Chemical Information and Modeling 2012;52:1621–36. doi:10.1021/ci200583t.
[24] Kubinyi H. QSAR: Hansch analysis and related approaches. Weinheim ; New York: VCH; 1993.

[25] Sun H, Nguyen K, Kerns E, Yan Z, Yu KR, Shah P, Jadhav A, Xu X. Highly predictive and interpretable models for PAMPA permeability. Bioorganic & Medicinal Chemistry 2017;25:1266–76. doi:10.1016/j.bmc.2016.12.049.
[26] Kansy M, Fischer H, Kratzat K, Senner F, Wagner B, Parrilla I. High-throughput artificial membrane permeability studies in early lead discovery and development. In: Testa B, van de Waterbeemd H, Folkers G, Guy R, editors. Pharmacokinetic Optimization in Drug Research, Verlag Helvetica Chimica Acta, Zürich, Switzerland; 2001, p. 447–64. doi:10.1002/9783906390437.ch24.
[27] Zhu C, Jiang L, Chen T-M, Hwang K-K. A comparative study of artificial membrane permeability assay for high throughput profiling of drug absorption potential. European Journal of Medicinal Chemistry 2002;37:399–407.
[28] Kerns EH, Di L, Petusky S, Farris M, Ley R, Jupp P. Combined application of parallel artificial membrane permeability assay and Caco-2 permeability assays in drug discovery. Journal of Pharmaceutical Sciences 2004;93:1440–1453.
[29] Galinis-Luciani D, Nguyen L, Yazdanian M. Is PAMPA a useful tool for discovery? Journal of Pharmaceutical Sciences 2007;96:2886–92. doi:10.1002/jps.21071.
[30] Chen X, Murawski A, Patel K, Crespi CL, Balimane PV. A novel design of artificial membrane for improving the PAMPA model. Pharmaceutical Research 2008;25:1511–20. doi:10.1007/s11095-007-9517-8.
[31] Wang CK, Northfield SE, Swedberg JE, Colless B, Chaousis S, Price DA, Liras S, Craik DJ. Exploring experimental and computational markers of cyclic peptides: Charting islands of permeability. European Journal of Medicinal Chemistry 2015;97:202–13. doi:10.1016/j.ejmech.2015.04.049.
[32] Oja M, Maran U. The permeability of an artificial membrane for wide range of pH in human gastrointestinal tract: experimental measurements and quantitative structure-activity relationship. Molecular Informatics 2015;34:493–506. doi:10.1002/minf.201400147.
[33] Oja M, Maran U. Quantitative structure–permeability relationships at various pH values for acidic and basic drugs and drug-like compounds. SAR and QSAR in Environmental Research 2015;26:701–19. doi:10.1080/1062936X.2015.1085896.
[34] Oja M, Maran U. Quantitative structure–permeability relationships at various pH values for neutral and amphoteric drugs and drug-like compounds. SAR and QSAR in Environmental Research 2016;27:813–32. doi:10.1080/1062936X.2016.1238408.
[35] Oja M, Maran U. pH-permeability profiles for drug substances: Experimental detection, comparison with human intestinal absorption and modelling. European Journal of Pharmaceutical Sciences 2018.
[36] Avdeef A, Bendels S, Di L i., Faller B, Kansy M, Sugano K, Yamauchi Y. PAMPA—critical factors for better predictions of absorption. Journal of Pharmaceutical Sciences 2007;96:2893–909. doi:10.1002/jps.21068.
[37] Stenberg P, Bergström CA, Luthman K, Artursson P. Theoretical predictions of drug absorption in drug discovery and development. Clinical Pharmacokinetics 2002;41:877–899.
[38] Worth A, Bassan A, Saliner AG, Netzeva T, Patlewicz G, Pavan M, Tsakovska I, Vracko M. The Characterisation of (Quantitative) Structure-Activity Relationships - Preliminary Guidance. EUR 21866, European Commission; 2005.
[39] Cruciani G, Crivori P, Carrupt P-A, Testa B. Molecular fields in quantitative structure–permeation relationships: the VolSurf approach. Journal of Molecular Structure: THEOCHEM 2000;503:17–30.

[40] Ruusmann V, Sild S, Maran U. QSAR DataBank repository: open and linked qualitative and quantitative structure–activity relationship models. Journal of Cheminformatics 2015;7:32. doi:10.1186/s13321-015-0082-6.
[41] Fischer H, Kansy M, Avdeef A, Senner F. Permeation of permanently positive charged molecules through artificial membranes—Influence of physico-chemical properties. European Journal of Pharmaceutical Sciences 2007;31:32–42. doi:10.1016/j.ejps.2007.02.001.
[42] Nakao K, Fujikawa M, Shimizu R, Akamatsu M. QSAR application for the prediction of compound permeability with in silico descriptors in practical use. Journal of Computer-Aided Molecular Design 2009;23:309–19. doi:10.1007/s10822-009-9261-8.
[43] Diukendjieva A, Alov P, Tsakovska I, Pencheva T, Richarz A, Kren V, Cronin MTD, Pajeva I. In vitro and in silico studies of the membrane permeability of natural flavonoids from Silybum marianum (L.) Gaertn. and their derivatives. Phytomedicine 2019;53:79–85. doi:10.1016/j.phymed.2018.09.001.
[44] Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. Journal of Medicinal Chemistry 2000;43:3714–7. doi:10.1021/jm000942e.
[45] Berthold MR, Cebron N, Dill F, Gabriel TR, Kötter T, Meinl T, Ohl P, Sieb C, Thiel K, Wiswedel B. KNIME: The Konstanz information miner. In: Preisach C, Burkhardt H, Schmidt-Thieme L, Decker R, editors. Data Analysis, Machine Learning and Applications, Springer Berlin Heidelberg; 2008, p. 319–26.
[46] Final Report Summary - COSMOS (Integrated in silico models for the prediction of human repeated dose toxicity of cosmetics to optimise safety). 2016.
[47] Karelson M, Karelson G, Tamm T, Tulp I, Jänes J, Tämm K, Lomaka A, Savchenko D, Dobcheva D. QSAR study of pharmacological permeabilities. ARKIVOC: Online Journal of Organic Chemistry 2009.
[48] Tulp I, Sild S, Maran U. Relationship between structure and permeability in artificial membranes: Theoretical whole molecule descriptors in development of QSAR models. QSAR & Combinatorial Science 2009;28:811–4. doi:10.1002/qsar.200860160.
[49] Abraham MH. Scales of solute hydrogen-bonding: their construction and application to physicochemical and biochemical processes. Chemical Society Reviews 1993;22:73–83. doi:10.1039/CS9932200073.
[50] Ruell JA, Tsinman O, Avdeef A. Acid-base cosolvent method for determining aqueous permeability of amiodarone, itraconazole, tamoxifen, terfenadine and other very insoluble molecules. Chemical and Pharmaceutical Bulletin 2004;52:561–565.
[51] Liu H, Sabus C, Carter GT, Du C, Avdeef A, Tischler M. In vitro permeability of poorly aqueous soluble compounds using different solubilizers in the PAMPA assay with liquid chromatography/mass spectrometry detection. Pharmaceutical Research 2003;20:1820–6.
[52] 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. European Journal of Pharmaceutical Sciences 2006;28:43–50. doi:10.1016/j.ejps.2005.12.008.
[53] Savić J, Dobričić V, Nikolic K, Vladimirov S, Dilber S, Brborić J. In vitro prediction of gastrointestinal absorption of novel β-hydroxy-β-arylalkanoic acids using PAMPA technique. European Journal of Pharmaceutical Sciences 2017;100:36–41. doi:10.1016/j.ejps.2017.01.005.