Predicting ADME properties *in silico*: methods and models

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Unfavourable absorption, distribution, metabolism and elimination (ADME) properties have been identified as a major cause of failure for candidate molecules in drug development. Consequently, there is increasing interest in the early prediction of ADME properties, with the objective of increasing the success rate of compounds reaching development. This review explores *in silico* approaches and selected published models for predicting ADME properties from chemical structure alone. In particular, we provide a comparison of methods based on pattern recognition to identify correlations between molecular descriptors and ADME properties, structural models based on classical molecular mechanics and quantum mechanical techniques for modelling chemical reactions.

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▼ The number of research papers, reviews and commercial products relating to the prediction of absorption, distribution, metabolism and elimination (ADME) properties of pharmaceutical compounds has increased dramatically in recent years [1–3]. The reason for this is that ADME properties have been recognized as a major reason for the failure of drug candidates in the late stages of drug development, with a large financial impact on the cost of R&D in the pharmaceutical industry [4].

The ability to model ADME properties of molecules will facilitate the drug discovery process in several ways. Compounds for high-throughput screening (HTS) are, at present, chosen primarily on the basis of potency. The ability to simultaneously filter these molecules *in silico* for suitable ADME properties will result in higher quality leads emerging from HTS campaigns. Probably the most widely used ADME 'model' is Lipinski's 'rule of 5', which is used in the pharmaceutical industry to filter out compounds likely to be purely absorbed through the human intestine, based on four simple rules related to molecular properties [5]. *In silico* models of ADME can also be used to test individual molecules or chemical libraries as 'license-in' opportunities, evaluating their suitability as potential drug molecules.

During lead optimization, the ability to predict the effect of a proposed structural modification *in silico*, before compound synthesis, will result in fewer redesign–synthesize–test cycles. This is most powerful if a model can provide guidance regarding the structural modifications that will improve a property.

Modelling ADME properties

ADME properties are difficult to model for several reasons. The quality of experimental data varies enormously and is often limited in quantity and chemical diversity. This is especially true in the case of data obtained directly from humans. In addition, ADME properties such as oral bioavailability, human intestinal absorption (HIA) and metabolic stability arise from multiple physiological mechanisms, which make them difficult to model with traditional quantitative structure–activity relationship (QSAR) approaches.

To deal with the biological complexity from which ADME properties arise, a range of different techniques must be applied. A schematic illustrating this spectrum is shown in Fig. 1. Models of ADME properties are most commonly based on empirical approaches such as QSAR. These techniques can be applied with great efficiency to large numbers of molecules, but require a significant quantity of high quality data from which to deduce a relationship between structure and activity. With increasing understanding of the structural basis of the mechanisms giving rise to ADME properties on a molecular level, approaches based on atomistic modelling will

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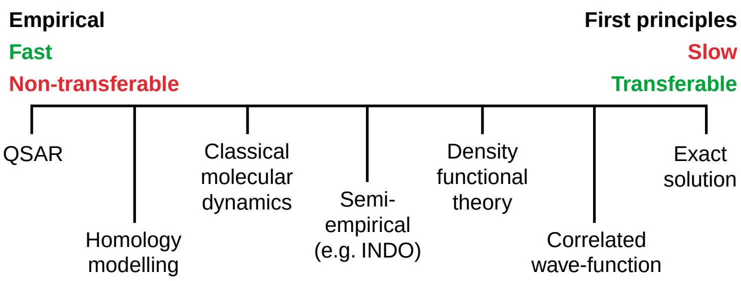

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Figure 1. The spectrum of approaches to modelling absorption, distribution, metabolism and elimination (ADME) properties. Trends across the scale are indicated by the words above. Advantages are highlighted in green and drawbacks in red. Methods to the left are empirical in nature, based on parameters fitted to large numbers of data points, whereas those to the right are based on first principle approaches. Empirical approaches tend to be faster, and able to make predictions many orders of magnitude faster than the first principle approaches which require large computational resources. Although empirical approaches may be applied to predict a wide range of properties, a large quantity of high-quality experimental data for each must be used for parameterization. By contrast, the first principle approaches are based on fundamental physical principles, and hence may be applied to a wide range of problems without re-parameterization.

become more widely applied. These techniques, based on molecular or quantum mechanics, are more computationally intensive, but make quantitative predictions based on general physical principles. The following sections will explore these approaches in greater detail and give examples of their application to the prediction of ADME properties of compounds.

Pattern recognition methods

The term ‘pattern recognition’ is used to describe any mathematical or statistical method that might be used to detect or reveal patterns in data. These identify a QSAR, relating a set of descriptors describing chemical structure (denoted X) to the observed activity (Y), which in this case is the ADME property of interest.

The parameters of the model relating Y to X are fitted from a set of molecules for which experimental data are available. This is commonly referred to as a ‘training set’. In general, models based on fitting approaches can only make reliable predictions for molecules similar to those in the training set – the ‘chemical space’ of the model. Although a prediction for a molecule significantly different from the training set might be correct, a source of uncontrolled error is introduced. The diversity of the molecules for which experimental data are available is the primary limiting factor in the applicability of models based on empirical fitting techniques.

Another difficulty in the fitting procedure is the avoidance of ‘over-fitting’. The fit of a model to the data in the training set might be improved by increasing the complexity of the model. However, improving the fit to the training set will eventually lead to a failure of the model to generalize to molecules that are not in this set. One approach for avoiding this is ‘cross-correlation’, whereby one or more molecules are omitted from the training set, and the model is refitted using the remaining molecules. Predictions for the omitted molecules are then made with the resulting model and the process repeated until predictions have been made for all of the molecules in the training set. The correlation coefficient between the predicted and experimental values is calculated (commonly referred to as Q^2) and compared with that for the model trained on the entire training set (R^2). No significant difference should be observed if the model is not over-fitted. A preferable approach, where sufficient data are available, is to use an independent test set of molecules to which the model is never fitted. If the model is not over-fitted, the correlation between predicted and experimental values for the test set should be comparable to that of the training set.

Several approaches can be used to train a QSAR model. The most common of these are briefly described in the following section. More details can be found in the references given [6–18].

Statistical regression

Statistical regression is primarily a tool for deriving linear models between X and Y, and has been applied in many ADME models, including the calculation of whole molecule physicochemical properties such as solubility and lipophilicity.

Multiple linear regression (MLR) is one of the oldest methods used to find a linear relationship between the observed activities and a set of descriptors [6]. A problem with this approach is that, as a rule of thumb, 4–5 examples (i.e. molecules) are required for each descriptor used. In addition, descriptors that are correlated or have skewed distributions – a common problem with chemical structures – will give poor regression models.

Partial least squares (PLS) has become a standard in this area. It overcomes most of the known problems with MLR, for example enabling the use of correlated descriptors [7,8]. A large number of descriptors can be used, even larger than the number of molecules in the training set, and the descriptors with the most influence on the model can be conveniently identified.

Simple non-linearities in the QSAR, for example, involving an exponent or logarithm of a descriptor value, can be easily included. More complex non-linear relationships can be treated by non-linear PLS [9]. However, other approaches are more suitable to modelling complex non-linear QSAR.

Classification methods

Where multiple biological mechanisms contribute to determining an ADME property, or a strongly non-linear relationship between the descriptors and property exists, it is often not possible to make a numerical prediction of that molecular property. An example of this is oral bioavailability, which is determined by absorption through the gut wall, possible active efflux by transporters such as P-glycoprotein, and metabolism in both the gut wall and liver.

In these cases, classification methods are often employed that assign the property of a molecule to one of two or more classes, rather than predicting a numerical value. Several techniques may be used to achieve this, including:

- Linear discriminant analysis (LDA) calculates discriminant functions or hyperplanes that partition the space of chemical descriptors to give the best separation between different classes [10].
- Decision trees create a branching structure in which the branch taken at each intersection is determined by a rule relating to the descriptors for a molecule. Each 'leaf' of the tree is assigned to a class [11].
- *k* Nearest Neighbours (*k*NN) identifies the *k* molecules in the training set that lie closest to the molecule for which the prediction is being made [12]. Distance is assessed using a measure of structural similarity, such as the Tanimoto index or an Euclidian distance in the space of descriptors [13]. The class of the unknown molecule is assigned by a voting procedure among the *k* nearest neighbours.

Artificial neural networks

Artificial neural networks (ANNs) is a pattern recognition approach inspired by the capabilities of the CNS [14]. A network of artificial neurones is 'trained' to reproduce Y given a set of inputs X for a training set of molecules. This network can then be used to make predictions for molecules for which Y is unknown.

ANNs are a powerful approach for fitting non-linear functions relating X and Y. However, it is often difficult to decode the final model to identify the changes to molecular structure needed to obtain a desired property. Also, ANNs have a tendency to 'memorize' rather than learn and are particularly susceptible to over-fitting, especially if the training data is noisy. Techniques such as 'regularization' can help to limit over-fitting, by penalizing overly complex functions during fitting [15].

Genetic algorithms

Genetic algorithms (GAs) are a class of heuristic search algorithms inspired by the mechanism of evolution [16]. The set of parameters defining a model relating Y to X represents the 'DNA' for an initial population with appropriate genetic diversity. A combination of 'mutation' and 'breeding' within this population is used iteratively to evolve the population and the 'fittest' of each generation is selected for the next iteration. The fitness of each model in the population is judged by the agreement between the predictions of the model and the data in a training set. A separate test-set guards against over-fitting.

GAs are sometimes used as an approach to training and designing ANNs [17]. Other forms of GA, such as genetic programming [18], can also generate non-linear relationships between descriptors and properties.

GAs are good at finding different solutions to the same problem, but the quality of the solutions are very sensitive to the parameters controlling cross-over and mutation rates.

Molecular and quantum mechanics

When an ADME property is influenced by the interaction of a compound with a molecular system, typically an enzyme or transporter, it is often possible to model directly the interaction at an atomistic level, enabling quantitative predictions to be made.

Chemical reactions, such as enzyme catalysis, require quantum mechanical (QM) simulations. However, the structural features of a complex formed by an interaction of a ligand with a binding site can be modelled using a molecular mechanics (MM) approach. In this approach, the atoms are treated with a classical 'ball and stick' approach. The principal advantage of MM lies in the computational efficiency of the method. However, the atomic interactions must be empirically parameterized for specific sets of atoms and it can be difficult to determine if the assumptions implicit in the parameterization are violated, which would introduce a source of uncontrolled error into the simulation.

The aim of QM methods is to obtain an approximate solution of the Schrödinger equation. Various approximations have been invoked for tractability, giving rise to *ab initio* and semi-empirical techniques. The most rigorous, and consequently the most computationally demanding, is *ab initio* theory. *Ab initio* methods use rigorous approximations borne from general physical principles alone and require no experimental parameters. As such, their quantitative application is not limited to specific types of system [19–21]. By contrast, experimental parameterization or neglect of the most time-consuming components of the Schrödinger equation gave rise to semi-empirical methods. Although not as reliable and versatile as *ab initio* techniques, such algorithms can be routinely applied to much larger systems [22–24].

To simulate the interaction of a ligand with a binding site, information regarding the structural basis for the interaction must be available. The lack of 3D structural data for proteins relevant to ADME processes is the main reason for the limited application of these techniques in this field. The exception to this is cytochrome P450 (CYP450), for which several crystal structures are available for bacterial (see for example [25–27]), as well as a single mammalian P450, CYP2C5 [28]. From these, homology models have been derived for some human P450s, including CYP2D6 [29] and CYP 3A4 [30]. As structural information on other drug metabolizing enzymes and transporters becomes available, the application of these techniques will become more widespread.

ADME models

Many models for ADME properties have been published, or are available commercially. Suppliers of commercial models include ArQule (Woburn, MA, USA) (formerly Camitro Corporation), Simulations Plus (Lancaster, CA, USA), Advanced Chemistry Development (Toronto, Ont., Canada), Amedis (Cambridge, UK), Accelrys (San Diego, CA, USA) and Lion Bioscience (San Diego, CA, USA).

Although this review does not give an exhaustive list of ADME models, an illustrative list of properties that have been modelled, including references for examples of published models, is described in the next section.

Whole molecule properties

Some physicochemical properties of molecules, although useful in their own right, provide important molecular descriptors for use in models of ADME properties. These models are typically derived via regression methods. In particular:

- Solubility. The Klopman solubility model is frequently quoted and widely used [31]. The training set for this model excluded molecules with charged atoms such as basic nitrogens or carboxylic acids. As many drug molecules contain charged atoms, the applicability of this model is limited. A model generated using an ANN has also been published by Liu and So [32].
- Acidity or basicity (p*K*a/p*K*b). This property is very important, as it determines the protonation state of the compound at different pH values.
- Hydrophobicity. This is based on measured octanol-water partition (logP) data. A commonly referenced model for logP is that of Moriguchi *et al.* [33]. This is a regression model built on a training set of 1230 compounds, for which it achieves a correlation of 0.952.

Human intestinal absorption

Wessel *et al*. have reported a model based on 76 compounds with reported HIA data, using GA with an ANN scoring function [34]. A standard error of 16% was obtained for the test set of 10 molecules.

Clark reports the use of polar surface area (PSA) to create a classification model to separate poor (<10%) from well-absorbed (≥10%) compounds [35], using the same data set as reported by Wessel *et al.* [34].

Egan *et al.* published a model for HIA based on PSA and logP descriptors alone [36]. This classification model was found to be between 74% and 92% accurate for different sets of molecules.

Abraham and his collaborators have recently reported a model based on a comprehensive HIA dataset [37]. Using 'Abraham descriptors', described in the same paper, the authors report $R^2 = 0.74$ when trained on the whole set. However, the authors also highlight the fact that the training set is heavily biased towards well-absorbed compounds (over 30% absorbed).

Blood–brain barrier

Young *et al.* have reported a model based on only six H_2 antagonists for which C_{brain}/C_{blood} were determined [38].

Abraham *et al.* have built a model based on 45 data points and experimentally determined descriptors, including the hydrogen-bonding capability of the molecule [39]. The use of descriptors that can only be obtained by an experimental method implies serious limitations in its use. However, a recent paper that calculates Abraham descriptors has made them available to the rest of the computational chemistry community [40].

A model based on 3D molecular descriptors and a discriminant PLS approach was published by Crivori *et al*. [41]. The resulting model was found to correctly predict more than 90% of the available blood–brain barrier (BBB) permeation data.

Human bioavailability

Hirono *et al*. [42] have built a model based on 188 drug molecules using the 'fuzzy adaptive least squares' method [43]. The model was validated by 'leave one out' cross validation, not an independent test set.

Yoshida and Topliss have reported a non-linear model, based on 232 compounds as their training set and 40 as a test set [44]. All the compounds have human bioavailability (%F) data reported, but the set was divided into four classes and discriminant functions were developed to separate the classes.

Andrews *et al*. have reported a model based on stepwise regression and 591 compounds [45]. Poor bioavailability predictions were false in only 3% of cases, but 53% of predictions of high bioavailability were incorrect.

Metabolism

Korzekwa *et al*. successfully applied semi-empirical QM methods to prediction of the regioselectivity of CYP450-mediated hydroxylation [46]. Using simple models for the haem-oxygen-thiolate molecule, the group modelled hydrogen abstraction from different carbons for a series of substrates. A correlation was observed between the stability of the product radicals with respect to their parents and the regioselectivity of CYP450 hydroxylation.

Harris and Loew studied the regioselectivities of metabolism of several substrates by P450$_{cam}$, a bacterial CYP450 from *pseudomonas putida*, using dynamical MM (molecular dynamics) simulations based on crystal structures of the substrate–enzyme complexes [47]. The aim of this study was to identify the sites of hydrogen abstraction from the substrate, thought to be the rate-limiting step in product formation. These simulations do not take into account the reactivity of each site on the substrate, which is calculated using QM approaches as described above. However, the results from MD identify those hydrogen atoms that are accessible to the reactive ferryl oxygen centre. The calculated regioselectivites agree well with those observed experimentally. Similar simulations, based on homology models, have been performed for human CYP3A4 [48] and CYP2C9 [49] enzymes.

Concluding remarks

No single approach can be used to predict the full range of ADME properties that are desired. A challenge in this field is to identify the technique that is most suitable for modelling the property under investigation. In fact, a combination of two or more models for the same property, based on different principles, can give higher confidence in the results obtained for which they agree or identify areas of uncertainty where they differ.

At present, models are available both in the literature and from commercial sources for the prediction of ADME properties such as solubility, HIA, bioavailability, BBB penetration and regioselectivity of CYP450 metabolism. Within the next year, the range of models will expand to include metabolism by other drug-metabolizing enzymes, active transport by proteins such as P-glycoprotein, plasma protein binding and renal and biliary clearance, among others. This will achieve coverage of the major ADME properties affecting the pharmacokinetics of compounds.

Physiologically based pharmacokinetic (PBPK) simulations have been developed (see for example [50,51]) that predict PK properties such as plasma concentration as a function of time, which, in turn, will yield parameters such as half-life and clearance. Existing simulations usually rely on compound ADME data obtained *in vitro* or *in vivo*. However, the results of predictive ADME models will be used as inputs to PBPK simulations, enabling accurate *in silico* prediction of PK properties from compound structure alone within the next two to three years. With this, compounds with unsuitable PK will be eliminated early in drug discovery and those designed with optimal properties will proceed into development [3].

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