Research Article

Neuro-fuzzy Models as an IVIVR Tool and Their Applicability in Generic Drug Development

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Abstract. The usefulness of neuro-fuzzy (NF) models as an alternative *in vitro-in vivo* relationship (IVIVR) tool and as a support to quality by design (QbD) in generic drug development is presented. For drugs with complicated pharmacokinetics, immediate release drugs or nasal sprays, suggested level A correlations are not capable to satisfactorily describe the IVIVR. NF systems were recognized as a reasonable method in comparison to the published approaches for development of IVIVR. Consequently, NF models were built to predict 144 pharmacokinetic (PK) parameter ratios required for demonstration of bioequivalence (BE) for 88 pivotal BE studies. Input parameters of models included dissolution data and their combinations in different media, presence of food, formulation strength, technology type, particle size, and spray pattern for nasal sprays. Ratios of PK parameters $C_{\max}$ or AUC were used as output variables. The prediction performance of models resulted in the following values: 79% of models have acceptable external prediction error (PE) below 10%, 13% of models have inconclusive PE between 10 and 20%, and remaining 8% of models show inadequate PE above 20%. Average internal predictability (LE) is 0.3%, and average external predictability of all models results in 7.7%. In average, models have acceptable internal and external predictabilities with PE lower than 10% and are therefore useful for IVIVR needs during formulation development, as a support to QbD and for the prediction of BE study outcome.

KEY WORDS: bioequivalence; *in vitro-in vivo* correlation (IVIVC); modeling; neuro-fuzzy; quality by design (QbD).

INTRODUCTION

In vivo data represent a difficult environment for modeling. The most important challenges in modeling are limited and expensive databases, high variability of data, and their high complexity. The consequence is that it is often difficult to build *in vitro-in vivo* correlation (IVIVC) which is preferred and suggested by regulatory agencies (1,2). For drugs with complicated pharmacokinetics, especially those with regional dependent absorption or pH dependent dissolution, for immediate release (IR) drugs or nasal sprays, suggested level A correlations are not capable to satisfactorily describe the IVIVC.

On the other hand, the need to predict pharmacokinetic behavior of IR drug formulations with active pharmaceutical ingredients (API) of different biopharmaceutics classification system (BCS) classes often arises during generic drug development. In that cases, we meet with a challenge while

dissolution data are not always sufficient to describe *in vivo* behavior of drugs and consequently, for majority of drugs we need to develop more complex models than level A, B, C, or multiple level C correlation, that base on *in vitro* dissolution data only (1). For lack of suggested/published models useful for substances with complicated pharmacokinetics and formulations different than extended release formulations and due to the need to have IVIVR, the usage of alternative method for development of IVIVR was unavoidable.

During the development of different models for IVIVR, it was recognized that often it is necessary to combine different dissolution media, numeric and text variables, to enable inclusion of all expensive and sparse *in vivo* data into a model. In this instance, we can use artificial intelligence modeling to combine inputs considered as reliable for *in vivo* prediction and thus, develop alternative IVIVR models with more information and formulation properties included (particle size for poorly soluble substances, dissolution in gastric and intestinal media, and prandial conditions in a bioequivalence (BE) study etc.).

Pieces of evidence of applicability of artificial intelligence models for solving problems in pharmaceutical sciences have been presented in published literature (3–7). Although fuzzy logic is less popular in comparison to neural networks, its transparency and possibility to add prior knowledge in the model give them preference especially when data for

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modeling are sparse and insight into the input/output relations is mandatory. Based on the literature and results of our previous work with neural networks and hybrid neurogenetic and neuro-fuzzy (NF) models used for pharmacokinetic (PK) parameter predictions (8,9), we recognized the advantages NF models could offer for IVIVR predictions.

Due to their properties, NF models seemed a reasonable option to solve the mentioned problems. The novel approach was statistically evaluated with 144 NF models for BE outcome predictions. The acceptable external predictability of models enables their use as an IVIVR tool.

Establishing an IVIVR, although less robust than an IVIVC, may be sufficient to assure product quality when combined with product and process understanding (10). With established IVIVR, we can evaluate the impact of formulation and process variable changes on drug product quality during development, predict the performance of the commercial batches based on the BE data from the exhibit batch manufactured at the pilot scale, and facilitate the evaluation of post-approval changes (10).

One of the opportunities where scientific progress could accelerate the development of generic products while maintaining high standards for quality, safety, and efficacy is the use of quality by design to develop bioequivalent products (11). Quality by design (QbD) identifies characteristics that are critical to quality from a safety and efficacy perspective, translates them into the attributes that the drug product should possess, and establishes how the critical process parameters can be varied to consistently produce a drug product with the desired attributes. To use QbD to develop a product that is bioequivalent to a reference product, a generic sponsor must have an understanding of what attributes of the formulation and manufacturing process have the potential to change the bioavailability of a particular active ingredient (11). Modeling and simulation play an important role in detection of mentioned attributes and also in determination of their limits that could lead us to a bioequivalent product.

Enhanced knowledge of *in vitro* and *in vivo* behaviors of the drug leads to a more rational clinical study design and relevant specifications on either product attributes or *in vitro* release methods (12). In this way, the number of generic formulations that need to be produced and also the number of unsuccessful BE studies are reduced. In generic drug development, accurate BE outcome predictions are very important, while expensive, extensive, or unpredictable BE studies can limit the development of a generic product (11). Relating to this, the applicability of NF models for the predictions of BE study outcome is presented in the continuation.

MATERIALS AND METHODS

The modeling process involved building hybrid artificial intelligence systems—NF models with the use of commercial software Neuframe version 4.0 (Neosciences 2000).

Neuro-Fuzzy Systems

NF systems belong to a group of artificial intelligence models together with neural networks, genetic algorithms, fuzzy logic, and other similar systems. Mentioned artificial intelligence methods are not competitive but synergistic and

complementary. Neural nets allow the easy creation of models from training sets; on the other hand, fuzzy logic system provide a powerful technique to create models that deal with the uncertainties prevalent in the real world. Although both fuzzy and neural approaches possess remarkable properties when employed individually, there are great advantages of using them synergistically. NF nets combine features of neural nets and fuzzy systems to provide a hybrid between the two techniques (13,14). Basic principles of fuzzy logic are defined in the continuation.

In classical set theory, there is a strict sense of membership to a set; that is, an element either belongs or does not belong to the set. In fuzzy sets, many degrees of membership are allowed. The degree of membership to a set is indicated by a number in the interval [0,1], and we no longer have crisp sets but fuzzy sets. Thus, infinite degrees of membership are possible, and we say that a *membership function* $\mu_A(x)$ maps every element of the universe of discourse X to the interval [0,1] as presented in Eq. 1 (13).

$$\mu_A(x) : X \rightarrow [0, 1] \quad (1)$$

There are several classes of parameterized functions commonly used to define membership functions: triangular, trapezoidal, Gaussian, generalized bell, and sigmoidal (15). As a form of membership function, piecewise polynomials of order k (basis spline (B-spline) basis functions) could be used (16–18). For order $k=2$, the basis function has the triangular shape used in many fuzzy logic implementations. The parameters associated with each basis (fuzzy membership) function are its order and the knot vector on which it is defined. The knots determine the size of the intervals and hence, define the width of each fuzzy set on the original input space (19). The introduction of additional knots within the basis functions enables increasingly complex functions to be approximated, while an increase in their order enables smoother functions to be obtained (20). A step that converts a crisp number to a fuzzy set is called *fuzzification* (13). Model variables are fuzzified to be partial members of membership functions or fuzzy sets in the interval grade (0,1) (16).

NF networks use the fuzzy logic system to store the knowledge acquired between a set of input variables ($x_1, x_2, \dots, x_n$) and the corresponding output variable (y) in a set of linguistic fuzzy rules that can be easily interpreted, such as IF x_1 is high AND x_2 is low, THEN y is high, $c=0.9$, where ($c=0.9$) is the rule of confidence which indicates how much the above rule has contributed to the output (16). Fuzzy *if/then* rules and their aggregations, known as *fuzzy algorithms*, are fuzzy relations in linguistic disguise. A relation implies the presence of an association between elements of different sets. In a case the degree of association is either 0 or 1, we have crisp relations, but if the degree of association is between 0 and 1, we have fuzzy relations. Fuzzy relations in fuzzy approaches possess the computational potency and significance that functions possess in conventional approaches (13). After the inputs are processed by the fuzzy algorithm, the result is a fuzzy output, and a crisp number is obtained with a process of *defuzzification* (13). The mean of maxima and center of gravity are the most popular defuzzification algorithms (16,17).

The modeling with Neuframe version 4.0 (Neusciences 2000) was performed based on B-spline NF networks that are trained with adaptive spline modeling of observation data (ASMODO) algorithm.

A typical structure of a NF network contains three layers: an input layer, a single hidden layer, and an output layer as presented in Fig. 1 (16,17,19,20). The input layer normalizes the input space in a p -dimensional lattice. Each cell of the lattice represents similar regions of the input space. The hidden layer consists of basis functions, such as B-spline functions, which are defined on the normalized input space (16,19). When B-splines are used to implement the fuzzy membership functions, generally, a singleton fuzzifier is chosen and defuzzification is usually done using a center of gravity algorithm (17). The output layer sums the weighted outputs from the basis functions according to Eq. 2:

$$y = \sum_{i=1}^p a_i w_i \quad (2)$$

y output

a_i output from the p th basis function

w_i connection weight associated with a_i (16).

To overcome the curse of dimensionality, the analysis of variance (ANOVA) representation is used to decompose an n -dimensional function into a linear combination of a number of subfunctions often called subnetworks:

$$f(x) = f_0 + \sum_{i=1}^n f_i(x_i) + \sum_{i=1}^{n-1} \sum_{j=i+1}^n f_{i,j}(x_i, x_j) + \dots + f_{1,2,\dots,n}(x) \quad (3)$$

where f_0 =function bias and other terms represent the univariate, bivariate, and high-order subfunctions (16,19).

For training of associative memory network (AMN)—a B-spline neurofuzzy network— instantaneous learning rules are formulated by minimizing instantaneous estimates of a performance function, which is generally the mean square output error (MSE), and the parameters (weight vector) are updated using gradient descent rules. The AMN built depends linearly on a set of weights which are updated using

Fig. 1. B-spline neuro-fuzzy network structure with four subnetworks and B-spline basis functions of second and third order (17,19)

the basic learning rules (19). Instantaneous learning rules only update those weights which contribute to the output, and learning is local because dissimilar inputs are mapped to different weight sets. The aim of these training rules is parameter convergence (19).

This AMN provides a direct link between artificial neural networks and the fuzzy systems. From a fuzzy viewpoint, the univariate B-spline basis functions represent fuzzy linguistic statements, and multivariate fuzzy sets are formed using the product operator to represent fuzzy conjunction. This link enables the B-spline networks to be interpreted as a set of fuzzy rules and allows modeling and convergence results to be derived for the fuzzy networks. One of the advantages in using B-splines is that linear relationships can be encoded within the network's structure, and when this type of knowledge is unavailable, algorithms such as ASMODO may be used to discover it, thus providing the designer with valuable knowledge about the unknown process (19).

The ASMODO algorithm is an algorithm for empirical modeling. It uses B-splines to represent general nonlinear and coupled dependencies in multivariable observation data. In the ASMODO algorithm, the output variable is modeled as a sum of several low dimensional submodels, where each submodel only depends on a small subset of the input variables. The decomposition of the high-dimensional input space into low-dimensional additive subspaces makes the model more transparent to the user, at the same time as the complexity (number of parameters) of the model is dramatically reduced (21). The ASMODO algorithm can perform input/output data mappings, similar to the way multilayer perceptrons (MLPs) do, with the additional benefit of being able to translate the acquired knowledge into a set of fuzzy rules that describe the model input/output relationships in a more transparent fashion (19). The internal structure of the model is, through an incremental refinement procedure, automatically adapted to the dependencies observed in the data. Only input variables which are found of relevance are included in the model, and the dependency of different variables is decoupled when possible. This makes the model more parsimonious (21).

Beside predictions of IVIVCs, artificial intelligence systems are used in many areas of pharmacy and medicine: molecular modeling, predictions of formulation composition, predictions of PK parameters, predictions of conditions and optimization of manufacturing process, QSAR studies, diagnosis of different illnesses, etc. (3,4). For PK parameters' predictions, neurogenetic models that are based on neural networks and genetic algorithms have the advantage of NF models in sense of lower prediction error, but the transparency of included knowledge into the system increases the applicability of NF models (9).

The advantages of NF models are the following (8,22):

- Provide a simple way to arrive at a definite conclusion based upon vague, ambiguous, imprecise, and noisy input information.
- Model is empirically-based.
- Enables modeler to include knowledge into a system and combine it with the acquired model knowledge.
- NF models are useful for modeling of complex, nonlinear systems also by offering the possibility to combine different numeric and text variables. In this way, we were able to include more formulation properties considered as

reliable for *in vivo* prediction in a model (particle size, dissolution in gastric and intestinal media, prandial conditions in a BE study, etc.).

- The obtained knowledge of NF system is transparent in opposition to neural networks where the knowledge is stored as weights of connections between neurons.

There are also some limitations of NF models:

- The result of the knowledge is not a mathematical equation but a set of fuzzy rules.
- Overfitting could occur in a case of limited databases.
- Reliable database is crucial for the quality of the model.
- In case of high complexity, the learning process could have a long duration.

With Neuframe version 4.0, NF-supervised learning nets were built. In supervised learning, the net creates a model that maps between a training input row and the required response as defined by the corresponding training target row. Supervised learning can be used wherever the desired response to a set of input values is known (14).

Data Used for Modeling

Models were primarily intended for the predictions of BE study results, and consequently, *in vivo* part of the database used for modeling consisted of PK parameters obtained with BE studies performed at Lek d.d. as well as literature data of BE studies performed with marketed formulations for instances when internal data were insufficient. BE studies were performed for different markets and included 45 test drug formulations with 23 different active pharmaceutical ingredients.

Models were built based on the importance of PK parameter for the success of BE study. For formulations where $C_{\max}$ ratio or area under the curve (AUC) ratio or both were defined as the most critical parameters for the success of BE study, only $C_{\max}$ or only AUC or both $C_{\max}$ and AUC models were built, respectively. Altogether, 36 models were built for $C_{\max}$ predictions only and 4 models only for AUC predictions. When both $C_{\max}$ and AUC were defined as critical for the success of BE study and while AUC models have lower percent prediction error (PE%) in comparison to $C_{\max}$ models, for majority of BE studies, both $C_{\max}$ (52 models) and AUC (52 models) were predicted to lower the risk for prediction failure. In some occasions, the same formulation was included in BE studies for different scopes and four formulations were fixed combinations. In this way, the total number of 144 models (88 for $C_{\max}$ and 56 for AUC) comes from combinations of API, formulations, $C_{\max}$, and AUC models.

Mostly, formulations were immediate release oral formulations, some of them were delayed or prolonged release and few of them were nasal sprays. Drugs used for modeling were members of different BCS classes. In majority, they belonged to BCS 2 and in minority, to BCS 3 class (Fig. 2). BCS class was defined based on in-house data of solubility and permeability or based on published data (23).

Fig. 2. Frequency distribution of APIs according to BCS classes

Each model was unique regarding the number and type of inputs that were chosen based on API and formulation properties in a way that prior knowledge was included in a model. Input parameters of models included dissolution data and their combinations in different media, BE study prandial design, formulation strength, technology process (i.e., different excipients used, wet granulation vs direct compression), particle size, and spray pattern for nasal sprays. Selection of dissolution media and methods depended upon formulation properties (i.e., low amount of surfactant for poorly soluble compounds). The number of input variables varied from 2 to 9 for all 144 models. Regarding the number of inputs, models for $C_{\max}$ and AUC predictions have the same median value of 6. All 88 $C_{\max}$ and 56 AUC models have included in average 5.6 and 5.8 inputs, respectively. There was no correlation between the number of inputs and prediction performance of models.

Removing inputs can sometimes be achieved at the cost of making the remaining model more complex (24).

Ratios of PK parameters $C_{\max}$ or AUC were included in models as output variables. The number of BE studies used for building the models varied from 2 to 9 and for testing from 0 to 7. After building and testing the models, BE study outcome was predicted before the subsequent BE study. External predictability of models was calculated after we had received the BE studies results. In total, 144 predictions of $C_{\max}$ and AUC ratios required for demonstration of BE for 88 pivotal BE studies with generic and reference drugs were performed with mentioned IVIVR NF models.

The Modeling Process

Each drug formulation and API requires a unique modeling approach regarding the input data and the purpose we would like to achieve, but in general, modeling process followed the next steps:

- Step 1 Building the database for learning and testing the model
- Step 2 Building the models, calculation of internal predictability (learning error (Le%))
- Step 3 Testing the models, calculation of test predictability (PE%^t), where applicable
- Step 4 Selection of the robust model with the minimum of error

- Step 5 Simulation with new data, prediction of BE study outcome
- Step 6 Validation of the model based on the results of the BE study, calculation of external predictability (PE%)

For some models, we did not dispose of enough data to test the model before the prediction of BE study outcome. For these cases, the step 3 was omitted.

To build the NF model in Neufuzzy module of Neuframe, we started with automatic network construction (ASMOD algorithm). In this type of training, an NF net based on the shape of the training data is created automatically. The user can choose between the following statistical significance measures: structural risk minimization, minimum descriptor length, leave one out cross validation, generalized cross validation, Akaike information criterion, Bayesian information criterion, and final PE. Failure margin (the number of times the rule base can get worse before training stops) and maximal subnet inputs should also be defined. The maximal subnet input is a value that limits the number of inputs used in a single rule. There is no need to scale inputs in Neufuzzy module of Neuframe (14).

The ASMOD procedure, which automatically determines the structure of B-spline network, is used to initialize fuzzy systems (19). When first placed on the workspace, the various parameters are given default values that automatically create a rule base from the training data (14). Usually, ASMOD starts with a very simple model structure, containing only a few prespecified submodel structures or none at all and iteratively refines the model structure during training so as to gradually increase model capability until some stopping criterion is met (16,21). The initial model is updated by evaluating the best refinement from a list of potential refinements:

- Pass 1 Univariate addition, tensor product, and tensor split
- Pass 2 Subnetwork deletion
- Pass 3 Knot insertion
- Pass 4 Knot deletion
- Pass 5 Reduce order (20)

With refinement passes, we can see the process used to create and test the rule set (14). The learning time for automatic network construction was below 1 min for all models and was not the time limiting step during the modeling procedure.

Once Neufuzzy has created a network structure using automatic network construction feature, the expert may take control over the structure by switching the training mode to weight rule confidence. In this mode, Neufuzzy uses the training data to recalculate the weights for the chosen network structure, without modifying the structure (14). We were able to change relevant inputs, B-spline order (shape), number and position of nodes, number of subnetworks, and fuzzy rules. In this way, prior knowledge about the process was incorporated in the design of the network. For the majority of models, we used membership functions of the triangular shape (B-spline order $k=2$). Typical B-spline NF network structure used is presented in Fig. 1.

Prediction Performance of Models

According to FDA Guidance for Industry (1), evaluation of internal predictability is based on the initial data used to define the IVIVC model, and evaluation of external predictability is based on additional test data sets. For internal predictability, average absolute PE% of 10% or less for C_{max} and AUC establishes the predictability of the IVIVC. In addition, the absolute PE% for each formulation should not exceed 15%. External predictability involves using the IVIVC to predict the *in vivo* performance for a formulation with known bioavailability that was not used in developing the IVIVC model. PE% of 10% or less for C_{max} and AUC establishes the external predictability of an IVIVC. PE% between 10 and 20% indicates inconclusive predictability and the need for further study using additional data sets. PE% greater than 20% generally indicates inadequate predictability, unless otherwise justified (1).

The MSE is an estimator of the accuracy of the model, and as such is often employed within significance measures (20). During the modeling, MSE and one additional chosen statistical significance measure were presented. The lower the MSE value the better the fuzzy rule set is in providing the correct answer to the data (14). While a network can always reduce the MSE by introducing additional degrees of freedom, additionally to the MSE, that has already been incorporated in a learning procedure as a performance function and was a basis for model selection, PE% was calculated manually for each selected model. Based on PE%, a final model for BE studies prediction was chosen. PE% gives us more transparent information about the model performance and is also suggested by FDA Guidance for Industry (1).

From predicted *versus* the measured differences of PK parameter ratio values, absolute (abs) learning and PE% was calculated for validation of learning and prediction performance of models:

PE% (external predictability):

$$PE\% = [\text{abs}(\text{Observed value} - \text{Predicted value}) / \text{Observed value}] \times 100$$

LE% (internal predictability):

$$LE\% = [\text{abs}(\text{Observed value} - \text{Predicted value}) / \text{Observed value}] \times 100$$

Internal and external predictability of models were calculated and evaluated based on the currently valid Guidance for Industry (1). Models with external PE of 20% and more were considered inadequate in sense of predictability and models with PE below or equal 10% were considered as acceptable. While in our case, calculated external PE% relates to the test and the reference drug ratio of C_{max} and AUC instead of the absolute PK parameters values the criteria for acceptable prediction performance of models are even more strict in comparison to the guideline, where PE of 10% for absolute values could result to PE of 20% for the ratio and still considered adequate. Statistical evaluation was performed with Minitab® 16.1.1, © 2010 Minitab Inc. and Excel, part of Microsoft Office Professional Plus 2012, © 2010 Microsoft Corporation.

RESULTS

To evaluate the new modeling approach, it was important to obtain sufficient sample of models to enable statistical evaluation. Applicability of hybrid artificial intelligence models is presented in detail with one model example for $C_{\max}$ and AUC predictions and additionally, with statistical evaluation of a sample of 144 models to demonstrate general predictability and usefulness of NF IVIVR models.

An Example of Neuro-fuzzy IVIVR Model

An IVIVR model for a BCS 2 class drug incorporated in a mono and combo formulations is presented as an example of IR drug, where combination of dissolution data, formulation strength, and particle size was necessary to gain acceptable predictability. Database used for this model consisted of eight BE studies performed for different formulation strengths and scopes in fasting conditions, which is the proposed condition according to the Guidance for Industry (1). A validated NF model for prediction of $C_{\max}$ and AUC ratios was built with the use of commercial software Neufame version 4.0 (Neosciences 2000).

BCS 2 class drugs are poorly soluble, therefore, it is expected that particle size is important parameter for *in vivo* dissolution and consequently for drug absorption. Correlation between particle size of test formulations and PK parameters ratios measured in 12 BE studies intended for different scopes are presented in Fig. 3. Determination coefficients for linear regression curves of particle size and PK parameters yield to values of 0.74 and 0.71 for $C_{\max}$ and AUC, respectively. These values indicate that relation between independent and dependent variables exists, but there are also other influences that should be included in the model to obtain the acceptable predictability. Linear regression models for particle size do not take into consideration the properties of the reference formulations taken from different markets. For this reason, we decided to combine the information obtained from the dissolution profiles of test and reference drugs with the particle size of test formulations to build the appropriate model.

The BE studies used for modeling included formulations of different strengths that is why strength was also added to input variables. All inputs of the model were the following: ratio of % dissolved of test and the reference drug at data points of 120, 150, and 180 min, particle size $d(0.5)$, and the formulation strength. $C_{\max}$ and AUC ratios of test and the reference drugs were used as outputs.

Dissolution data for modeling were obtained from dissolution testing method using the following conditions: apparatus 2 at 50 rpm in 1,000 ml of 0.01 M HCl with addition of Tween 80 and 13 mM of potassium ions. The dissolution results for 120, 150, and 180 min data points for test, and reference product are presented in Table I. The input and output minimum values used for model training equals 0. The origin was included in training to obtain more robust models for extrapolations, while although predicting the extrapolated values is not desirable, at least some extrapolations of training measured data often cannot be avoided. Large extrapolations of measured data used for training introduce high risk of prediction failure; therefore, we need to avoid them. Values of inputs/outputs used for modeling are presented in a Supplementary Table S I.

Results of eight BE studies intended for different scopes were used for model training, while the validation of the model was performed with two BE studies, and additionally, one BE study was predicted with a validated model. The database used for modeling and modeling results together with internal and external predictability are presented in Table II.

According to the results in Table II, learning error for $C_{\max}$ and AUC predictions is 4.0 and 3.3%, respectively. Model was validated with two BE study results, with average external prediction error of 3.7% for $C_{\max}$ and 5.1% for AUC. Calculated average and individual PE% and LE% for $C_{\max}$ and AUC are below 9%, which is acceptable according to the Guidance for Industry (1) and confirms adequate predictability of the model.

The model was used to predict formulation 5, included in a BE study. Predicted values of $C_{\max}$ and AUC ratios (94.5 and 95.3%, respectively) of test and reference drugs are close to the measured values of $C_{\max}$ and AUC ratios (99.6 and 96.1%, respectively) obtained

Fig. 3. Linear relationship between particle size of test products and $C_{\max}$ or AUC ratio of test and the reference formulation

Table I. Percent Dissolved (Q) in 120, 150, and 180 min for Test and Reference Products in a Model Example

Formulation no.	Sample	Q 120 min	Q 150 min	Q 180 min	Used for
1 ^m	Test	84.6	87.2	88.7	Model training
	Reference	74.5	79.6	81.6	
2 ^c	Test	85.8	87.5	89.2	
	Reference	82.1	86.0	89.9	
3 ^m	Test	58.1	62.2	68.4	Model validation
	Reference	55.0	60.8	65.0	
3 ^m	Test	78.6	82.5	85.2	
	Reference	64.7	71.2	75.8	
4 ^c	Test	82.8	84.8	86.8	Model validation
	Reference	70.4	74.2	78.0	
4 ^c	Test	82.8	84.8	86.8	
	Reference	67.4	73.2	79.1	
6 ^c	Test	83.7	86.0	87.7	Model validation
	Reference	82.1	84.4	85.5	
6 ^c	Test	82.8	84.8	86.7	
	Reference	73.8	77.4	81.0	
3 ^m	Test	78.6	82.5	85.2	Model validation
	Reference	69.3	75.4	80.1	
4 ^c	Test	82.8	84.8	86.8	
	Reference	72.1	75.3	78.4	
5 ^m	Test	66.6	77.3	84.3	Prediction
	Reference	74.1	80.4	84.8	

Formulations 1–6 differ in strength and in number of APIs. All dissolution data refer to one API only
 c combination product—2 APIs in one formulation, m mono product

in a BE study, what additionally confirms adequate predictability of the model (prediction errors are 0.7 for AUC and 5.1% for C_{max}).

The structure of the model was selected in accordance with the lowest internal and external prediction error of the model and was based on the assessment of reasonable impact of inputs on the output values. Both C_{max} and AUC models have similar structure, with five subnetworks and one input connected to each subnetwork. Triangular membership functions (B-splines order 2) were used for inputs and outputs. B-spline membership functions together with the range of inputs are presented in the Supplementary Figure S 1. In Neufuzzy, input variables can be set to any order, but the output fuzzy variable is fixed with order 2. The structure of an NF model and obtained knowledge are presented below in a form of fuzzy rules for C_{max} and AUC relations with inputs:

C_{max} model

INTERNAL NETWORK 1 WITH 5 SUBNETWORKS

SubNetwork1 with 1 Input Variable and the 2 following Fuzzy Rules:

- 1 IF d(0.5) is low, THEN c_{max} is low (0.69) OR c_{max} is high (0.31)
- 2 IF d(0.5) is high, THEN c_{max} is low (0.70) OR c_{max} is high (0.30)

SubNetwork2 with 1 Input Variable and the 2 following Fuzzy Rules:

- 3 IF dissolution at 120 min is low, THEN c_{max} is low (0.65) OR c_{max} is high (0.35)

- 4 IF dissolution at 120 min is high, THEN c_{max} is low (1.00)

SubNetwork3 with 1 Input Variable and the 2 following Fuzzy Rules:

- 5 IF dissolution at 150 min is low, THEN c_{max} is low (0.80) OR c_{max} is high (0.20)
- 6 IF dissolution at 150 min is high, THEN c_{max} is high (1.00)

SubNetwork4 with 1 Input Variable and the 2 following Fuzzy Rules:

- 7 IF dissolution at 180 min is low, THEN c_{max} is low (0.65) OR c_{max} is high (0.35)
- 8 IF dissolution at 180 min is high, THEN c_{max} is low (0.98) OR c_{max} is high (0.02)

SubNetwork5 with 1 Input Variable and the 2 following Fuzzy Rules:

- 9 IF strength is low, THEN c_{max} is low (0.69) OR c_{max} is high (0.31)
- 10 IF strength is high, THEN c_{max} is low (0.71) OR c_{max} is high (0.29)

AUC model

INTERNAL NETWORK 2 WITH 5 SUBNETWORKS

SubNetwork1 with 1 Input Variable and the 2 following Fuzzy Rules:

- 1 IF d(0.5) is low THEN AUC is low, (0.64) OR AUC is high (0.36)
- 2 IF d(0.5) is high THEN AUC is low, (0.64) OR AUC is high (0.36)

SubNetwork2 with 1 Input Variable and the 2 following Fuzzy Rules:

- 3 IF dissolution at 120 min is low, THEN AUC is low (0.58) OR AUC is high (0.42)
- 4 IF dissolution at 120 min is high, THEN AUC is low (1.00)

SubNetwork3 with 1 Input Variable and the 2 following Fuzzy Rules:

- 5 IF dissolution at 150 min is low, THEN AUC is low (0.73) OR AUC is high (0.27)
- 6 IF dissolution at 150 min is high, THEN AUC is high (1.00)

SubNetwork4 with 1 Input Variable and the 2 following Fuzzy Rules:

- 7 IF dissolution at 180 min is low, THEN AUC is low (0.61) OR AUC is high (0.39)
- 8 IF dissolution at 180 min is high, THEN AUC is low (0.79) OR AUC is high (0.21)

SubNetwork5 with 1 Input Variable and the 2 following Fuzzy Rules:

- 9 IF strength is low, THEN AUC is low (0.64) OR AUC is high (0.36)
- 10 IF strength is high THEN AUC is low, (0.66) OR AUC is high (0.34)

Table II. Prediction Performance of the Model Example

Formulation no.	Particle size d(0.5)	C _{max} ratio% measured	C _{max} ratio% predicted	C _{max} LE%	AUC ratio% measured	AUC ratio% predicted	AUC LE%
Training data	1 ^m	115.0	109.1	5.1	107.9	103.2	4.4
	2 ^c	107.7	105.4	2.2	97.8	96.7	1.1
	3 ^m	78.4	79.5	1.4	80.7	81.8	1.4
	3 ^m	103.8	110.1	6.0	95.5	100.0	4.7
	4 ^c	106.7	116.1	8.8	99.1	106.1	7.0
	4 ^c	119.9	115.5	3.6	104.2	100.7	3.4
Average learning error Formulation no.	6 ^c	90.2	89.0	1.4	87.4	85.8	1.9
	6 ^c	104.3	100.6	3.5	94.2	92.2	2.1
Average learning error							
Validation data	Particle size d(0.5)	C _{max} ratio% measured	C _{max} ratio% predicted	C _{max} PE%	AUC ratio% measured	AUC ratio% predicted	AUC PE%
	6.3	111.8	107.1	4.2	103.3	97.6	5.3
Average validation error Formulation no.	4.9	111.2	114.8	3.2	101.5	106.4	4.8
	6.8	99.6	94.5	3.7	96.1	95.3	5.1
Predictions				5.1			0.7

Formulations 1–6 differ in strength and in number of APIs. All predictions refer to one API only
c combination product—2 APIs in one formulation, m mono product

From fuzzy rules for d (0.5) and strength, little or no impact on the output is noticed. While during the input data analysis, we recognized possible impact of both inputs to the prediction of the output (Fig. 3), and while their impact on the output is in line with the expected correlations (negative correlations for both inputs), we decided to keep them in the model despite minor impact on the model output. Due to the low solubility of drug, these correlations were in accordance to our prior knowledge and therefore kept with the model, while when generating nonlinear models, removing inputs can sometimes be achieved at the cost of making the remaining model more complex (24).

Negative correlations observed with some dissolution time points can indicate a complex *in vivo* behavior of BCS 2 class drug: variable dissolution, precipitation, regional dependent metabolism, or interactions between them, but due to relatively small databases, that are usual for IVIVC predictions, some correlations could be the result of fitting the noise. Therefore, fuzzy rules should be interpreted with caution.

Additional examples of models for all BCS classes are presented in the Supplementary material to the manuscript.

Statistical Evaluation of General Predictability of Neuro-fuzzy IVIVR Models

Success of a BE study is mostly dependent on C_{max}, due to its sensitivity on formulation and physiological changes and consequently higher variability in comparison to AUC. This is the reason that C_{max} predictions are the most important challenge for modeling. Statistical evaluation of external predictability (PE%) for all the models and separately for C_{max} and AUC models is presented in Table III. For majority of BE studies, we predicted both C_{max} and AUC to lower the risk of prediction and consequently BE study failure.

Average internal predictability of all models is 0.3%. Average external predictability of all 144 models is 7.7% with a median of 5.5%. It means that in general models, predictability is acceptable and below required 10% (Fig. 4). Median value is less impacted by the individual high prediction errors and is therefore more informative regarding the overall predictability. As expected, due to the higher variability, average PE% for C_{max} models is higher (8.9%) in comparison to AUC models (5.6%). Also, median external prediction error values follow this trend: 6.6 and 4.3% for C_{max} and AUC models, respectively. All prediction errors of models meet the range of 0.01 to 34.4% (Table III).

Regarding the percentage of acceptable models, we can conclude that 79.2% or 114 out of 144 models have

Table III. Basic Statistics of External Predictability of All Models

PE %	All models	C _{max} models	AUC models
Mean	7.7%	8.9%	5.6%
Median	5.5%	6.6%	4.3%
Min	0.01%	0.20%	0.01%
Max	34.4%	34.4%	28.0%
N of models	144	88	56

Fig. 4. External predictability of all models

acceptable predictability with PE% below 10% (Fig. 4). At $\alpha=0.05$, we were not able to confirm normal distribution of log PE% (p value <0.001) with Ryan-Joiner test (25). For this reason, both parametric and nonparametric tests were performed for models evaluation.

One sample t test was performed to test whether average PE% of all $C_{\max}$ and AUC models equals to the desired 10% or it is higher than 10%. The null (H_0) and alternative hypotheses (H_1) were the following: $H_0: \mu \leq 10$; $H_1: \mu > 10$. Based on $\alpha=0.05$ and the p values of 1.0, 0.887, and 1.0 for all $C_{\max}$ and AUC models, respectively, we did not reject the null hypothesis. The p values were higher and conclusions were the same with log PE% values tested instead of PE%.

Due to the nonconfirmed normality, Wilcoxon signed-rank test was performed to test whether median PE of all $C_{\max}$ and AUC models equal to 7% or it is higher than 7%. While the median PE% limit is not determined in Guidelines, value of 7% was defined based on $C_{\max}$ models predictability, where median PE% of 6.6% gives us a mean PE% value of 8.9%, which is acceptable according to Guidelines (1). The null and alternative hypotheses were the following: H_0 median PE% ≤ 7 ; H_1 median PE% > 7 . Based on $\alpha=0.05$ and the p values of 0.935, 0.257, and 1.0 for all $C_{\max}$ and AUC models, respectively, we did not reject the null hypothesis. The p values were higher and conclusions were the same with log PE% values tested instead of PE%.

Inconclusive external predictability of 10–20% is calculated for 12.5% or 18 models out of 144. In addition, 8.3% or 12 models out of 144 have inadequate external predictability above >20% (Fig. 4).

Based on a separate evaluation of $C_{\max}$ and AUC models, we can conclude that 70.5% or 62 out of 88 $C_{\max}$ models have acceptable predictability below 10%. Inconclusive predictability of 10–20% is noticed in 18% or 16 models out of 88. In addition, 11.4% or 10 models out of 88 show inadequate predictability above >20% for $C_{\max}$ (Fig. 5). On the other side, 92.9% or 52 out of 56 AUC models have acceptable predictability below 10%. Inconclusive predictability of 10–20% is noticed in 3.6% or 2 models out of 56. In addition, 3.6% or 2 models out of 56 show inadequate predictability above >20% for AUC (Fig. 5).

Impact of BCS Classification on Modeling Ability

The biopharmaceutical classification system is a framework for classifying drugs based on their aqueous solubility and intestinal

Fig. 5. External predictability of $C_{\max}$ and AUC models

permeability (26,27). In general, the most appropriate BCS class for building IVIVC is class 2, due to dissolution rate-limited absorption. IVIVC is not likely for BCS 1 and 3 class drugs in IR formulations, where gastric emptying and permeability rate-limited absorption is noticed, respectively. There is also low possibility of IVIVC for BCS 4 class drugs (26). In some cases, possibility of modeling could also increase for drugs that belong to unsuitable BCS classes for modeling. This could be achieved by modifying the release of the formulation and by the impact of excipients and API on *in vivo* permeability, solubility, dissolution, and gastrointestinal tract (GIT) motility. With the impact on the GIT region of absorption, the limiting factor for absorption could be changed.

Drugs we used for modeling were members of different BCS classes (Table IV). Mean external prediction errors for individual class were the following: 6.9, 8.4, 2.9, 7.6, and 10.4% for BCS 1, 2, 3, 4, and for class with undefined permeability (BCS 2 or 4), respectively. All BCS 1 class drugs we used for modeling were incorporated in modified release formulations, which increased the importance of drug release for drug

Table IV. PE% for $C_{\max}$ and AUC Models According to BCS Classification

PE%	BCS 1 ^a	BCS 2	BCS 3	BCS 4	BCS 2 or 4
Mean	6.9%	8.4%	2.9%	7.6%	10.4%
Median	5.9%	7.5%	1.8%	4.6%	6.9%
Min	0.01%	0.8%	0.4%	0.2%	0.7%
Max	23.5%	34.4%	6.9%	31.7%	28.2%
N of models	36	53	7	40	8

^a All BCS 1 class drugs were incorporated in modified release formulations

absorption and enabled modeling. One-way ANOVA was performed with BCS class as a factor to define statistical differences of PE% among BCS classes. With Levene's test, p value of 0.356 and alpha 0.05, the assumption of equal variances has been met. With p value of 0.273 and alpha 0.05, we cannot reject the H_0 about equality and we can conclude that there are no statistical differences in PE% between different BCS class drugs. Graphically, this is presented with box plots in Fig. 6, and we can also notice that high prediction errors of models are rather outlier than average values.

Additionally, nonparametric Kruskal–Wallis ANOVA with alpha 0.05 and p value of 0.122 gives us the conclusion of equality of medians of PE% between different BCS class drugs. For BCS 3 class drugs, the PE% is the lowest (2.9%), but this could be a random occurrence due to the low number of models and APIs for this class in comparison to other BCS classes (Fig. 2, Table IV).

DISCUSSION

For many drug formulations, it is often a challenge to build IVIVC preferred for extended release formulations by guidance (1,2), while in many cases dissolution or release in one medium is not the only limit for drug absorption. In example, for poorly soluble drugs with regional dependent absorption and/or metabolism along GIT, it is important that similar amount of test and reference drug is dissolved in stomach and in GIT to be available for absorption at the same region of GIT. At least two dissolution media should be included in a model to describe such *in vivo* behavior. To develop IVIVR intended for BE outcome predictions, we need to describe and detect the differences between the test and the reference drug that are tested in the same subjects with similar physiological conditions and basic API properties. Furthermore, predictions have to meet the mean of all subjects included in a study, and not all of them have the same drug absorption rate limited step. In such instances, we are forced to combine dissolution profiles and possibly also other *in vivo* relevant numerical and text inputs.

With their properties, NF models were recognized as an alternative and reasonable method in comparison to the published approaches for IVIVC and preferential for solving

of complex IVIVR problems. As inputs, only root causes of differences in test and the reference drug *in vivo* behavior need to be included in a model. In this way, models are simplified. With learning, data also sets the knowledge of *in vivo* differences in test and the reference drug is incorporated in the model. NF technique enables the usage of combinations of text and numerical data and thus, combinations of different dissolution data in one model in opposition to the known methods that build IVIVC based on a single dissolution medium only. With the predictions of $C_{\max}$ and AUC ratios of test and reference drugs instead of absolute values like in other known IVIVC models, the combinations of BE study data obtained in different bioanalytical laboratories are enabled, and thus, the modeling database is increased. While *in vivo* data are very expensive, this can save money for additional BE data that would be needed for modeling approach according to the current Guidelines for IVIVC.

Beside the acceptable external predictability of NF models, their usefulness was additionally confirmed with comparison to the physiologically based pharmacokinetic (PBPK) model built with GastroPlus™ (Simulations Plus, Inc., Copyright 2001–2012). Both physiological and NF models intended for the same BE study prediction were built in parallel. The prediction ability of both models was similar (PE of 1.8 and 1.4% for NF and GastroPlus™ models, respectively). This comparison in prediction performance should be additionally confirmed statistically with higher number of models built with both techniques where applicable. Properties of different IVIVC approaches are presented in the Supplementary Figure S 2.

While 79.2% of models have acceptable predictability with external PE below 10%, 8.3% have inadequate predictability—above 20%. Reasons for failure of NF models predictions are usually the changes in formulation or manufacturing process, changes in API, differences between reference formulations from different markets, errors in analytics or measurements of input/output variables, lack of the data and overfitting, nonpredictive dissolution methods, or *in vivo* impact of different excipients, not observed *in vitro*. Average internal predictability yields to 0.3% due to small databases and high fitting abilities of NF models. Due to high possibility of overfitting, external predictability is the only reliable parameter for evaluation of IVIVR NF models. As for all kind of models, the quality of input data is crucial to build a useful NF model for IVIVR, what requires teamwork of different experts and inclusion of large amount of knowledge on drug formulations and API to select the proper inputs. With a possibility to choose different inputs, models are useful for prediction of many kinds of API and formulation properties.

Despite the best possibility of building IVIVR for BCS 2 class drugs, in our case, such drugs have the highest PE% (8.4%) among all BCS classes. Although this higher PE% is statistically not significant in comparison with PE% of other BCS classes, models for BCS 2 class drugs are very sensitive to selection of dissolution media, especially in cases where dissolution behavior of test and reference drugs changes with the media, which can increase the risk of prediction failure. Based on the presented results, all BCS class drugs are worth to be modeled with NF models during drug development, although the decision of modeling should be accepted based

Fig. 6. Box plot of PE% for drugs in different BCS classes. Class 5 includes drugs with undetermined permeability (BCS class 2 or 4). White triangle—median PE% value, white square—mean PE% value, asterisk—outlier values

on a case-by-case evaluation of *in vivo* relevant inputs. In case of unsuccessful attempts to develop a model despite evidence that permeability or gastric emptying are not rate-limited factor for absorption, our attention should be focused on possibility of incorrectly defined inputs. In this way, NF modeling can facilitate the finding of *in vivo* predictive dissolution methods and critical quality attributes needed for QbD.

CONCLUSION

NF models also proved a useful alternative IVIVR tool for drugs with complicated PKs where relations between input and output variables are complex and nonlinear, and our mathematical understanding of the system is incomplete. It should be stressed that reliable database is crucial for the quality of the model and a lot of expertise should be introduced to adequately define inputs that are unique for each formulation.

The acceptable external predictability of models enables their use as an IVIVR tool for the predictions of BE study outcome during drug development and consequently, facilitates the determination of important factors for rate and extent of drug absorption. This can lead to fast and accurate decisions for start of BE studies. Due to mentioned properties, NF models could support decisions when they need to be taken based on incomplete data and are also applicable to QbD approach in generic drug development. An outcome is the reduced number of developed generic formulations and performed BE studies and consequently faster and lower-priced development.

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