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Generalized regression neural networks in prediction of drug stability

Svetlana Ibrić, Milica Jovanović, Zorica Djurić, Jelena Parojčić, Ljiljana Solomun and Branka Lučić

Abstract

This study had two aims. Firstly, we wanted to model the effects of the percentage of Eudragit RS PO and compression pressure as the most important process and formulation variables on the time course of drug release from extended-release matrix aspirin tablets. Secondly, we investigated the possibility of predicting drug stability and shelf-life using an artificial neural network (ANN). Ten types of matrix aspirin tablets were prepared as model formulations and were stored in stability chambers at 60°C, 50°C, 40°C and 30°C and controlled humidity. Samples were removed at predefined time points and analysed for acetylsalicylic acid (ASA) and salicylic acid (SA) content using stability-indicating HPLC. The decrease in aspirin content followed apparent zero-order kinetics. The amount of Eudragit RS PO and compression pressure were selected as causal factors. The apparent zero-order rate constants for each temperature were chosen as output variables for the ANN. A set of output parameters and causal factors were used as training data for the generalized regression neural network (GRNN). For two additional test formulations, Arrhenius plots were constructed from the experimentally observed and GRNN-predicted results. The slopes of experimentally observed and predicted Arrhenius plots were tested for significance using Student's *t*-test. For test formulations, the shelf life ($t_{95\%}$) was then calculated from experimentally observed values ($t_{95\%}$ 82.90 weeks), as well as from GRNN-predicted values ($t_{95\%}$ 81.88 weeks). These results demonstrate that GRNN networks can be used to predict ASA content and shelf life without stability testing for formulations in which the amount of polymer and tablet hardness are within the investigated range.

Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia

Svetlana Ibrić, Milica Jovanović, Zorica Djurić, Jelena Parojčić, Branka Lučić

Hemofarm A.D, Beogradski put b.b., 26300 Vršac, Serbia

Ljiljana Solomun

Correspondence: Svetlana Ibrić, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia.
E-mail: ibric@beotel.yu

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Introduction

A pharmaceutical formulation is composed of several formulation factors and process variables. Numerous responses relating to the usefulness, drug release and stability, as well as safety, must be optimized simultaneously. This is called a multi-objective optimization problem. Where an explicit physical model cannot be built because of unclear correlations between causal factors and responses, mathematical methods such as artificial neural networks (ANNs) may be useful. An ANN is an intelligent non-linear mapping system built to loosely simulate the functions of the human brain. An ANN model consists of many nodes and their connections. Its capacity is characterized by the structure, transfer function and learning algorithms (Lippmann 1987; Erb 1993). Because of their model independence, non-linearity, flexibility, and superior data fitting and prediction ability, ANNs have gained interest in the pharmaceutical field in the past decade. ANNs have been used to solve various problems such as product development (Takayama et al 2000; Ibric et al 2002; Plumb et al 2002; Kachrimanis et al 2003), quantitative structure–activity relationships (Aoyama et al 1990; Agrafiotis 2002; Huuskonen 2003), quantitative structure–pharmacokinetic relationships (Hussain et al 1993; Gao et al 2002), estimation of diffusion coefficients (Jha et al 1995), prediction of the permeability of skin (Lim et al 2002; Degim et al 2003) and Caco-2 cells (Fujiwara 2002), and prediction of mechanisms of drug action (Weinstein 1992). However, there are no reports in the literature of using ANNs to predict the drug stability and shelf life of pharmaceutical formulations.

In our previous study, we used an ANN to optimize drug release from an extended-release aspirin formulation (Ibric et al 2002). Eudragit RS PO (the matrix substance) and

compression pressure (expressed as tablet hardness) were identified as the most important (causal) factors responsible for cumulative percentage of aspirin released in 8 h. An optimization method using a generalized regression neural network (GRNN) was used to define extended-release matrix aspirin tablets with optimum release behaviour. The optimum solution estimated with GRNN was formulation with 2.5% Eudragit RS PO and tablet hardness of 60 N. Release profiles predicted by the GRNN correlated well with experimental values. This satisfactory prediction by the GRNN of drug release for test and 'optimal' formulations clearly shows the applicability of GRNN in modelling extended-release tablet formulations.

The objective of the current study was to evaluate the effect of the percentage of Eudragit RS PO as matrix substance and compression pressure (i.e. tablets hardness) on the time course of drug release from the formulation, and to investigate whether an ANN can be used to predict drug stability and shelf life.

Materials and Methods

Materials

Acetylsalicylic acid (ASA) was Ph. Eur. grade. Eudragit RS PO was kindly supplied by Rhőm Pharma (Darmstadt, Germany); Avicel PH 112 (FMC Co., Philadelphia, PA, USA); Aerosil 200 (Degussa, Frankfurt, Germany), Pruv (JRS Pharma, Rosenberg, Germany) and talc (Ph. Eur grade) were used in model formulations.

Preparation of aspirin tablets

As model formulations, ten kinds of ASA tablets were selected according to the two-factor spherical second-order central composite design (Table 1). The amounts of Eudragit RS PO (X_1) and compression pressure, expressed through tablet hardness, (X_2) were selected as causal factors. The amount of ASA was fixed at a value of 325 mg per tablet (tablet weight 400 mg). The amount of Aerosil 200, Pruv and talc were fixed at 2, 4 and 6 mg per tablet, respectively.

Tablets were prepared using a direct-compression method. All ingredients were weighed accurately according to the experimental design and mixed well in a polyethylene bag. Flat-faced punches with a diameter of 10 mm were used to compress the powder mixture, using an eccentric compressing machine (EKO Korsch, Berlin, Germany). Tablet hardness was measured using a Erweka TBH 28 hardness tester (Erweka GmbH, Heusenstamm, Germany). Values presented are the average of 20 measurements.

Trials were performed in random order. All of the ingredients used in this study came from the same batches, and the same procedures and equipment were used throughout the production and testing of the tablets.

Table 1 Central composite design: factors and responses

Factors	Levels used				
	$-2^{1/2}$	−1	0	+1	$+2^{1/2}$
X_1 (% Eudragit RS PO)	2%	2.58%	4%	5.41%	6%
X_2 (tablet hardness, N)	25	35	57.5	80.4	90

Responses

$Y_1 = k_{60}$ (rate constant of apparent zero-order kinetics at 60°C)
$Y_2 = k_{50}$ (rate constant of apparent zero-order kinetics at 50°C)
$Y_3 = k_{40}$ (rate constant of apparent zero-order kinetics at 40°C)
$Y_{4=}\ k_{30}$ (rate constant of apparent zero-order kinetics at 30°C)

Stability testing

All formulations were made on the same day in controlled ambient conditions (20°C, 20% relative humidity), packed in brown-glass containers with a desiccant and stored in stability chambers at different temperatures: 60°C, 50°C, 40°C or 30°C, under controlled humidity (10%). Samples were taken at predefined time points and the ASA and salicylic acid (SA) content was measured using stability-indicating HPLC. During the stability test, the ASA and SA content in tablets was measured every week for 4 weeks for samples stored at 60°C, every week for 6 weeks for samples stored at 50°C, at 2, 4, 6, 8, 10 and 12 weeks for samples stored at 40°C, and every month for 6 months for samples stored at 30°C.

HPLC procedure

The HPLC procedure used the external standard method, and areas under the peaks were used for calculations. We used an Econosil RP-18, 250×4.6 mm column (Alltech Associates, Inc., Deerfield, IL, USA) and an injection volume of 20 μL. The mobile phase was a 45:55 mixture of acetonitrile and water (pH 2.5) delivered at a flow rate of 1.5 mL min^{-1}. Detection was at 230 nm.

Data analysis

Potencies (C) of aspirin as a function of the time (t) were constructed for each formulation, at each temperature (T). The results obtained fitted the function C = f (t,T), indicating that the decrease in aspirin content follows apparent zero-order kinetics ($r^2 > 0.9$). Constants of apparent zero-order kinetics for each temperature were chosen as the output variables for the ANN ($Y_1 = k_{60}$, $Y_2 = k_{50}$, $Y_3 = k_{40}$, $Y_4 = k_{30}$). Test formulations (T1 and T2) were prepared to validate the predictive ability of the ANN (Table 2).

Computational methods

We used commercially available Statistica Neural Networks software (StatSoft, Inc., Tulsa, OK, USA) throughout the study. A GRNN was used for modelling and optimization of extended-release aspirin tablets. GRNNs were introduced by Specht in 1991 and estimate the most probable value for continuous dependent values of a given dataset. They promote the probability density functions of the given patterns and finally attribute to them the value to which they most belong (Specht 1991). GRNNs are feed-forward networks comprised of four layers. The input layer comprises a variable number of neurons, which is equal to the number of independent features

Table 2 Inputs and outputs used for network training (F1–F10) and network testing (T1 and T2)

Formulation	Inputs		Outputs			
	% Eudragit RS PO	Tablet hardness (N)	k_{60} (s^{-1})	k_{50} (s^{-1})	k_{40} (s^{-1})	k_{30} (s^{-1})
F1	5.41	35	0.975	0.8193	0.1408	0.1243
F2	6	57.5	1.548	0.8641	0.1867	0.1663
F3	2.58	80	1.424	0.7239	0.2269	0.2047
F4	5.41	80	0.824	0.4468	0.1999	0.1249
F5	4	90	1.739	0.6489	0.1353	0.0785
F6	2.58	35	0.917	0.5900	0.0811	0.0591
F7	4	57.5	1.109	0.6921	0.2196	0.1524
F8	4	57.5	1.110	0.6139	0.1995	0.1267
F9	2	57.5	0.954	0.6146	0.1637	0.1492
F10	4	25	1.265	0.8411	0.1106	0.1087
T1	2.5	60	0.877	0.6029	0.1578	0.1146
T2	5.5	30	1.053	0.6243	0.1642	0.1172

$k_{60/50/40/30}$ are the rate constant of apparent zero-order kinetics at 60/50/40/30°C.

the network is trained on. The normalized input vector is copied onto the pattern units in the pattern layer, each representing a training case. An exponential activation function is applied, and the corresponding activation level is forwarded to the summation unit, where the density estimate of each pattern of each group or possible value is summarized. Finally, Bayesian theory is used to define the fourth layer. The main advantage of GRNNs is that they involve a single-pass learning algorithm and are therefore much faster to train than the well-known back-propagation paradigm (Specht 1990). Furthermore, they differ from classic neural networks in that every weight is replaced by a distribution of weights. This enables a large number of combinations of weights to be explored, and the exploration is less likely to end in a local minimum (Bruneau 2001). Therefore, no test and verification sets are necessary and, in principle, all available data can be used for the network training. In a GRNN model, it is possible to select the number of units (nodes) in the second radial layer, the smoothing factor (which controls the deviation of the Gaussian kernel function located at the radial centres), and the clustering algorithm (e.g. subsampling, K-means or Kohonen).

Initially, in the radial layer, the number of hidden units varied from 1 to 10, using smoothing factor 0.1 and the K-means clustering algorithm. To select the optimal GRNN model, the observed versus predicted responses were shown in the regression plots drawn for the two test formulations, which were excluded from the ten-formulation data set. The GRNN model that yielded a regression plot with a slope and squared correlation coefficient (r^2) that was closest to 1.0 was selected as the optimal GRNN model. A sum-squared error function was used in the network training. (The error is the sum of the squared differences between the target and actual output value on each output unit.)

Learned GRNN was used for modelling, simulation and optimization of the model extended-release formulation in the following ways: testing experimental points in experimental fields; searching for the optimal solutions; presenting response surfaces (or contour plots). To ensure that the results of the trained network are real and that there are no artifacts of the training process, an external validation can be done by predicting the stability (i.e. ASA content) and shelf life for two additional test formulations. The results obtained experimentally can then be compared with those predicted by the network.

Results and Discussion

Stability study

The ASA and SA content of samples of tablets stored at different temperatures and controlled humidity was measured using stability-indicating HPLC. Changes in ASA and SA content in samples stored at 50°C and at 60°C for 4 weeks and in samples stored at 40°C and 30°C for 6 months are presented in Table 3.

The *US Pharmacopeia* monograph for aspirin extended-release tablets allows a variation in ASA content of 5%, and in SA as impurity of 3%. The results show that changes in ASA and SA content are within pharmacopoeia limits for all tablet formulations, except for formulation F5, where the ASA content was 93.99% after 4 weeks' storage at 60°C. (The SA content (2.43%) was acceptable.)

Minimal degradation of active substance for all tablet formulations and in all storage conditions is in accordance with the results published by Vachon and Nairn (1997, 1998). Electrostatic association of the drug with the charged quaternary residues in the polymer may be responsible for the observed stability of ASA.

GRNN structure

The two causal factors corresponding to different percentage of Eudragit RS PO (X_1) and tablet hardness (X_2) were used as each unit of the input layer. Constants of apparent zero-order kinetics were used as output layers (four). A set of outputs and causal factors was used as tutorial data and fed into the computer. A GRNN was chosen as the network type. Several training sessions were conducted using different numbers of

Table 3 Acetylsalicylic acid (ASA) and salicylic acid (SA) content after 4 weeks at 50°C and 60°C and after 6 months at 40°C and 30°C for the formulations (F1–F10) and test formulations (T1 and T2)

	50°C		60°C		40°C		30°C	
	ASA (%)	SA (%)	ASA (%)	SA (%)	ASA (%)	SA (%)	ASA (%)	SA (%)
F1	97.05 ± 0.06	0.750 ± 0.008	97.82 ± 0.03	2.21 ± 0.02	98.29 ± 0.08	0.850 ± 0.009	99.07 ± 0.03	0.427 ± 0.010
F2	97.32 ± 0.08	0.779 ± 0.009	95.17 ± 0.03	2.44 ± 0.01	97.56 ± 0.08	0.906 ± 0.010	97.35 ± 0.04	0.466 ± 0.012
F3	97.17 ± 0.06	0.663 ± 0.010	95.12 ± 0.04	1.84 ± 0.01	96.59 ± 0.07	0.817 ± 0.0085	96.64 ± 0.06	0.478 ± 0.010
F4	98.45 ± 0.05	0.712 ± 0.008	97.17 ± 0.05	3.15 ± 0.01	96.33 ± 0.08	0.886 ± 0.011	97.54 ± 0.06	0.403 ± 0.08
F5	97.27 ± 0.05	0.695 ± 0.010	93.99 ± 0.04	2.43 ± 0.02	97.7 ± 0.04	0.918 ± 0.010	98.87 ± 0.06	0.82 ± 0.07
F6	99.91 ± 0.08	0.652 ± 0.011	98.12 ± 0.05	2.42 ± 0.02	100.46 ± 0.05	0.706 ± 0.011	100.56 ± 0.02	0.365 ± 0.08
F7	98.12 ± 0.05	0.706 ± 0.012	97.30 ± 0.05	2.25 ± 0.02	97.08 ± 0.07	0.785 ± 0.009	98.76 ± 0.01	0.444 ± 0.09
F8	99.58 ± 0.04	0.711 ± 0.011	96.18 ± 0.06	2.18 ± 0.01	97.05 ± 0.06	0.788 ± 0.008	98.76 ± 0.03	0.455 ± 0.08
F9	98.45 ± 0.08	0.620 ± 0.009	97.43 ± 0.07	2.10 ± 0.01	97.86 ± 0.06	0.707 ± 0.012	97.76 ± 0.02	0.446 ± 0.07
F10	97.95 ± 0.07	0.612 ± 0.009	95.2 ± 0.05	1.70 ± 0.01	98.2 ± 0.08	0.790 ± 0.010	98.6 ± 0.04	0.339 ± 0.08
T1	98.96 ± 0.08	0.638 ± 0.008	98.34 ± 0.04	2.14 ± 0.01	98.56 ± 0.08	0.726 ± 0.009	99.09 ± 0.02	0.455 ± 0.09
T2	98.45 ± 0.09	0.767 ± 0.012	96.20 ± 0.06	2.22 ± 0.01	97.08 ± 0.08	0.901 ± 0.010	98.75 ± 0.04	0.385 ± 0.010

Data are mean ± s.d of three measurements

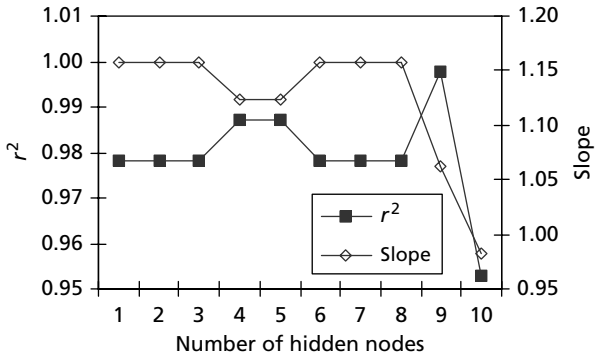

Figure 1 Plot of the regression slopes and squared correlation coefficients (r^2) for the two test formulations as a function of the number of hidden layer units using a GRNN model with 1–10 layer units.

units in the hidden layer in order to determine the optimal GRNN structure. Test data using the results from the two test formulations (T1 (2.5% Eudragit RS PO, 60 N) and T2 (5.5% Eudragit RS PO, 30 N)) were prepared to validate the prediction ability of the ANN.

Regression plots were drawn to show the predicted and observed responses for the two test formulations and the slopes and r^2 values determined. Figure 1 is a representative plot of the slopes and r^2 values for the GRNN model as a function of the units number of the hidden layer. On the basis of the data shown in Figure 1, the optimized GRNN model consisted of nine units in the radial hidden layer, since both the slope and r^2 values approached 1.0.

The learning period was completed when the minimum value of the root mean square (RMS) was reached:

$$\mathrm{RMS} = \left[\sum (y_i^{\,p} - y_i^{\,m})^2 / n\right]^{1/2}$$

where $y_i^{\,p}$ is the experimental (observed) response, $y_i^{\,m}$ is the calculated (predicted) response and n is the number of experiments. The selected ANN structure had four layers: the first layer had two input units, the second layer had nine hidden units (with negative exponential activation and radial postsynaptic function), the third layer had five units, and the fourth layer had four output units. Nine units in a hidden layer were needed to obtain an excellent prediction of the response variable.

GRNN training

Training set (inputs and outputs F1–F10, Table 2) was fed into the computer, and after assignment of radial units in the second layer of the GRNN using the K-means clustering algorithm, the predicted outputs were obtained (Table 4).

GRNN testing

For two test formulations, Arrhenius plots were constructed from experimentally observed and GRNN-predicted results. For the T1 formulation: experimentally observed $ln(k) = -5.8298\ 10^3/T + 17.101$ ($r = 0.956$) and GRNN-predicted $ln(k) = -5.7174\ 10^3/T + 16.705$ ($r = 0.959$). For the T2 formulation: experimentally observed $ln(k) = -6.2355\ 10^3/T + 18.471$ ($r = 0.944$) and GRNN-predicted $ln(k) = -6.1288\ 10^3/T + 18.06$ ($r = 0.953$).

Correlation plots of predicted versus experimentally obtained apparent zero-order constants for the test formulations (T1, T2) were constructed. Values for the correlation coefficients obtained were greater than 0.99 ($r^2 = 0.998$), indicating a strong correlation between the predicted and experimentally observed apparent zero-order constants for the test formulations.

The slopes of the experimentally observed and predicted Arrhenius plots were tested using Student's t-test. The observed values of $t_{test1} = 0.0269$ and $t_{test2} = 0.01378$ are lower than $t_{2,\ 0.05} = 2.920$), indicating that there was no significant difference between these plots. It further indicates excellent

Table 4 Predicted (P) and experimentally observed (E) values of apparent zero-order release constants for the formulations (F1–F10) and test compounds (T1 and T2)

	k_{60} (s^{-1})		k_{50} (s^{-1})		k_{40} (s^{-1})		k_{30} (s^{-1})	
	P	E	P	E	P	E	P	E
F1	1.120	0.975	0.8302	0.8193	0.1257	0.1408	0.1165	0.1243
F2	1.548	1.548	0.8641	0.8641	0.1867	0.1867	0.1663	0.1663
F3	1.424	1.424	0.7239	0.7239	0.2269	0.2269	0.2047	0.2047
F4	0.824	0.824	0.4468	0.4468	0.1999	0.1999	0.1249	0.1249
F5	1.739	1.739	0.6489	0.6489	0.1353	0.1353	0.0785	0.0785
F6	0.917	0.917	0.5900	0.5900	0.0811	0.0811	0.0591	0.0591
F7	1.109	1.109	0.6530	0.6921	0.2096	0.2196	0.1396	0.1524
F8	1.109	1.110	0.6530	0.6139	0.2096	0.1995	0.1396	0.1267
F9	0.954	0.954	0.6146	0.6146	0.1637	0.1637	0.1492	0.1492
F10	1.12	1.265	0.9302	0.8411	0.1257	0.1106	0.1165	0.1087
T1	0.954	0.877	0.6146	0.6029	0.1637	0.1578	0.1192	0.1146
T2	1.167	1.053	0.6976	0.6243	0.1641	0.1642	0.1290	0.1172

ability of the GRNN to predict ASA content for each formulation of matrix tablets with a polymer content of 2–6% and tablet hardness of 25–90 N.

Predicting the ASA content of the test formulation T1

In our previous paper (Ibric et al 2002), formulation T1 (2.5% Eudragit RS PO, 60 N) was chosen as the optimal formulation in terms of drug release. Using Arrhenius plots, constants of apparent zero-order kinetics of degradation at 20°C were calculated from experimentally observed values and from GRNN-predicted values. The shelf life was calculated to be 82.90 weeks from the experimentally observed values and 81.88 weeks from the predicted values.

Conclusion

The values for shelf life calculated from experimentally observed values and the GRNN-predicted values are very close, showing that there is no significant difference between GRNN-predicted and experimentally observed shelf life.

The satisfactory prediction of drug content for the training and test formulations by the GRNN in this study clearly shows the applicability of a GRNN in modelling the stability of extended-release tablet formulations. These results demonstrate that it would be possible, using GRNN, to predict drug content and shelf life for every formulation where the amount of polymer and the tablet hardness are within the investigated range, without performing additional stability testing.

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