RESEARCH PAPER

Accepted 16 May 2003
Revised 26 March 2003
Received 20 July 2002
Indian J. Pharm. Sci., 2003, 65(3): 274-278

Artificial Neural Network for the Simultaneous Estimation of Multicomponent Sample by UV Spectrophotometry

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A novel approach, using artificial neural networks, has been made to analyse multicomponent sample by UV spectrophotometry. Artificial neural network, based on back propagation paradigm, was custom developed to process the ultraviolet spectral data. A typical drug combination, diclofenac sodium and paracetamol, was chosen as model drug mix to evaluate the suitability of neural approach to quantify the components in a mixture. The ability of the artificial neural network to predict the concentrations was tested with several spectra of known ratios of the drugs under several neural network parameters. Our study indicated that artificial neural networks could produce accurate and precise results and could be used for routine analysis of multicomponent samples.

Artificial Neural Network (ANN) has become the focus of much attention in the past few years largely because of their scope of application in diverse areas, from missile technology to medical diagnosis[1]. ANN is a computation technique, composed of elements (artificial neurons) that perform in a manner that is analogous to the most elementary function of the biological neuron. ANN exhibits a surprising number of brain's characteristics; for example, they learn from experience, generalise from previous examples to new ones and abstract essential characteristics from inputs containing irrelevant data. The theory regarding neural network has been thoroughly reviewed[1-3]. Several studies have been reported in literature on application of ANN for determining the spectra-structure relationship[4-5], prediction of the secondary and tertiary structure of proteins[6-8], quantitative structure-activity relationships[9-10], prediction of physico-chemical properties of organic compounds[11], pharmaceutical product development[12-14], pharmacokinetic and pharmacodynamic analysis[15-16], pharmaceutical research[17-18], and diagnostic and clinical medicine[19-21]. In this study, the authors have custom developed a software package, "UV-Neural" in "C" programming language for Linux platform, for the UV spectrophotometric analysis of multicomponent samples by ANN technology and evaluated its performance using paracetamol (PAR) and diclofenac sodium (DIC) as model sample mix.

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MATERIALS AND METHODS

DIC and PAR were received as gifts from M/S MMC Healthcare Ltd., Chennai. The spectrophotometric reading were recorded on a Shimadzu 1601PC (Japan) double beam spectrophotometer controlled by UVPC software ver3.7. All the computation works were carried out using a Hewlett Packard brio personal computer with Pentium III 933 MHz and 128 MB RAM.

Neural computation:

The custom developed software package, UV Neural, could perform three functions; generate data required for training the ANN, train the ANN under user defined parameters to generalise the relation between the spectral data and sample composition using the training data and predict concentrations of sample components from their absorbance values at the selected wavelengths using the trained ANN.

ANN architecture:

The ANN employed in the present work was a feed forward network with three layers of artificial neurones, input, hidden and output layers. The number of neurones in each layer was n, 2n+1 and m in the input layer, hidden layer and output layer, respectively, where n is the number of wavelength points at which the absorbance values were measured for the analysis and m is the number of compónents in the sample. Each neuron is connected to the neurons in the adjacent layer. The hidden and output layers contain an additional input called the 'bias'. The input to a neuron in the hidden and output layers is computed by summing the product of each connection and the weight of that product. The value produced by the bias is added to the input, allowing the neuron to operate in the functional range optimal for mapping the inputs to output. The neuron activation function was then applied to the input to produce an output, which then serves as the input to the neurons in the next layer. In the present study sigmoid or hyperbolic tangent activation functions[2] were employed as the neuron transfer functions.

Each set of inputs and target constitutes a 'training pair'. In the present study the absorbance values of a multicomponent drug sample at selected wavelength points as the input and the corresponding concentrations of the individual drugs in the sample as target formed a training pair. The collection of all the training pairs representing absorbance values for various proportions of the sample mix constituted the training set. The inputs are fed to the network to produce a value at the output, which is compared with the target. The training process involved the adjustment of different weights, by an algorithm, towards finding a minimum difference between the output and the target value of the entire training set. The most popular back propagation algorithm[2] using the gradient descent principle was used to train the network. Each chosen neural network was trained till the sum squares, which was the square of the difference between the output and the target of the network was reduced to a predetermined level, called tolerance level, varying from 0.001 to 0.00005.

Training the ANN:

UV spectra of pure DIC and PAR solutions at a concentration of 10.0 μg/ml in 0.1M sodium hydroxide were recorded, individually, between 220 and 350 nm. The spectra were then converted to ASCII format using channel export function of the UVPC software controlling the spectrophotometer. The exported text file contains the absorbance val-

TABLE 1: TRAINING PARAMETERS FOR ANN OPTIMISATION.

To study the effect of	Parameters						
	Wave length range (nm)	No. of input neurons[1]	No. of training pairs	Weight initialisation	Learning rate	Activation function	Error tolerance
Wave length range	Varied	16	169	Random	0.1	Sigmoid	0.0001
No. of input neurons	295-220	4 to 16	169	Random	0.1	Sigmoid	0.0001
No. of training pairs	295-220	16	16 to 3721	Random	0.1	Sigmoid	0.0001
Weight initialisation	295-220	16	169	Random or Nugyen	0.1	Sigmoid	0.0001
Learning rate	295-220	16	169	Random	0.05 to 0.9	Sigmoid	0.0001
Activation function	295-220	16	169	Random	0.1	Sigmoid or nonsigmoid	0.0001
Error tolerance	295-220	16	169	Random	0.1	Sigmoid	0.0005 to 0.0001

ues against a series of wavelength in a two-column format. This file is then directly used by the custom developed UV-Neural software to create the training set, containing the required number of training pairs on the basis of training parameters prescribed interactively to the software programme. A number of different networks with different parameters (Table 1) were trained to study the effect of ANN parameters like nature of input (wavelength range), number of neurons (number of wavelength points), number of training pairs, weight initialisation, learning rate, activation function and the error tolerance to arrive at an optimum ANN that would perform best for the spectral problem at hand. Once the neural network arrives at the desired error tolerance, the synaptic weights are stored to a disk file constituting the knowledge set along with the respective configuration file.

Evaluation of ANN performance:

Spectral data for known mixture of DIC and PAR over a range of proportions (Table 2) were generated from the spectra of pure DIC and PAR obtained above by channel calculation function of the UVPC software. These spectral data were converted to ASCII format as above and used as test data. The performance of the trained ANN was evaluated by its ability to predict the concentration of PAR and DIC from the test data generated above accurately with acceptable precision by executing the UV Neural software in analysis mode. The results were subjected to statistical analysis. The accuracy was calculated as percentage theoretical value at 99% confidence limit (CL) and precision was measured in terms of standard error of means (SEM).

RESULTS AND DISCUSSION

DIC and PAR combination was chosen, as a model drug mix, for the reason that DIC has very low UV absorptivity compared to PAR (fig. 1) and they are combined in 1:10 ratio in drug formulations making conventional approaches for the estimation of the minor component by UV spectrometry difficult and challenging. Since the objective of the study was to determine the capacity of the ANN to determine the concentrations of individual components accurately in a multi-component formulation, experimental error had to be eliminated thus attributing any error to the ANN performance alone. Therefore simulated spectra generated, for different combinations of DIC and PAR (Table 2), were used to assess the ANN performance. Test data were deliberately chosen to differ from that used for the training the network in order to evaluate the generalisation capability of the network.

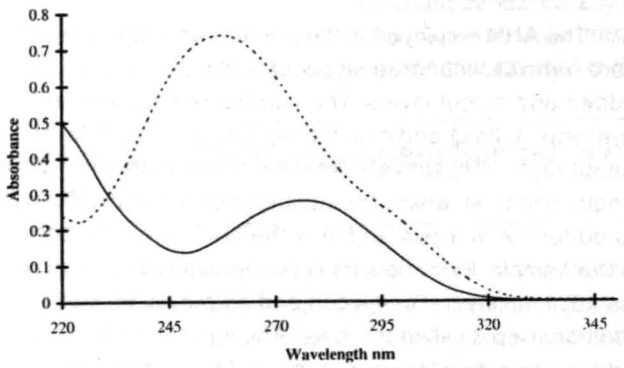

Fig. 1: UV Spectra of DIC and PAR.

Overlain spectra of 10 μg/ml solutions of DIC (—) and PAR (- - -) in 0.1M sodium hydroxide.

A crucial aspect of applying neural networks to a particular problem was arriving at optimal network parameters. To achieve this ANN was trained with various combinations of the parameters as given in Table 2. Satisfactory ANN performance was possible only when the wavelength range chosen covered the λ max regions of DIC and PAR, (fig. 2) Change in the number of wavelength points within this range did not affect the ANN performance (fig. 3). Studies revealed that the ANN could be trained only with sigmoidal and not by non-sigmoidal activation functions. It was observed that weight initialisation had no influence on ANN performance. As expected the accuracy and precision were improved when tolerance level was decreased (fig. 4). The optimum tolerance was found to be 0.0001, as further decrease did not improve ANN performance but increased the training time with marginal decline in performance. Learning rate did not influence the accuracy of the method but improved the pre-

TABLE 2: THEORETICAL CONCENTRATION OF PAR AND DIC IN TEST DATA.

Test Set No.		1	2	3	4	5	6	7	8	9
Concentration (μg/ml)	DIC	1.5	1.5	1.5	6.5	6.5	6.5	10.5	10.5	10.5
	PAR	2.5	6.5	10.5	2.5	6.5	10.5	2.5	6.5	10.5

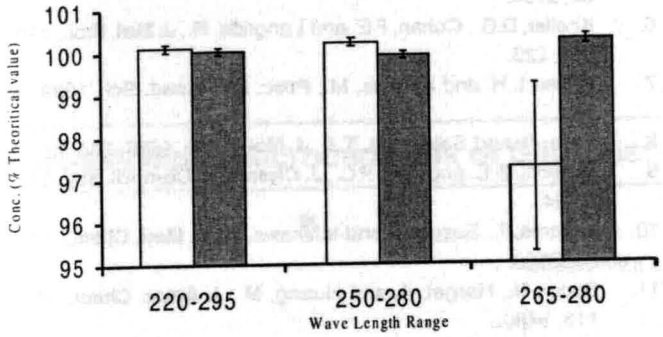

Fig. 2: Effect of wavelength range on ANN performance.

Effect of wavelength range on the accuracy of prediction of DIC (-□-) and PAR (-■-) concentrations by ANN with 16 input neurons, 169 training pairs, sigmoid activation function, random initialization, 0.1 learning rate and 0.0001 error tolerance.

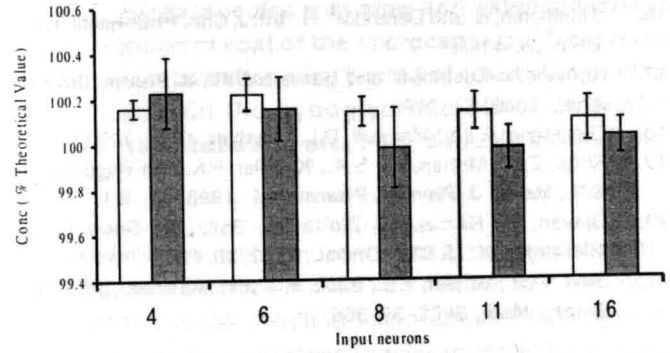

Fig. 3: Effect of number of input neurons on ANN performance.

Effect of number of input neurons on the accuracy of prediction of DIC (-□-) and PAR (-■-) concentrations by ANN with wavelength range 220 to 295 nm, 169 training pairs, sigmoid activation function, random initialization, 0.1 learning rate and 0.0001 error tolerance.

cision when it was decreased (fig. 5) down to 0.1; further decrease merely increased the learning time with out increase in precision. Besides it was also noted that the increase in number of training pairs improved ANN performance (fig. 6), however when it was above 441 there was no learning. Based on the above ANN performance studies it could be concluded that the optimum conditions for the analysis of DIC and PAR combination were 169 training pairs (encompassing concentration range 0 to 12 μg/ml for both the drugs), Random initialisation, binary sigmoid activation function, 0.1 learning rate, 0.0001 error tolerance and 16 input neurons (wavelengths from 220 to 295 nm with an interval of 5 nm). Under these conditions the ANN could be

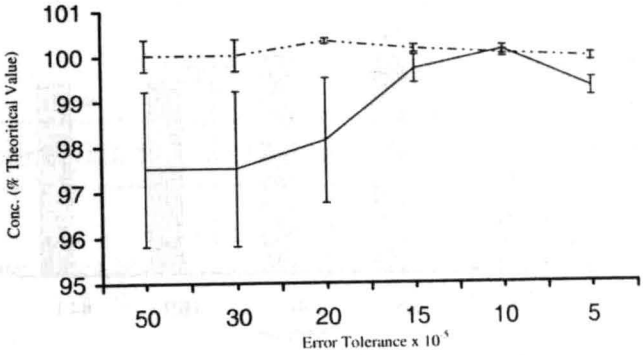

Fig. 4: Effect of error tolerance on ANN performance.

Effect of error tolerance on the accuracy of prediction of DIC (-□-) and PAR (-■-) concentrations by ANN with 16 input neurons, wavelength range 220 to 295 nm, 169 training pairs, sigmoid activation function, random initialization and 0.1 learning rate.

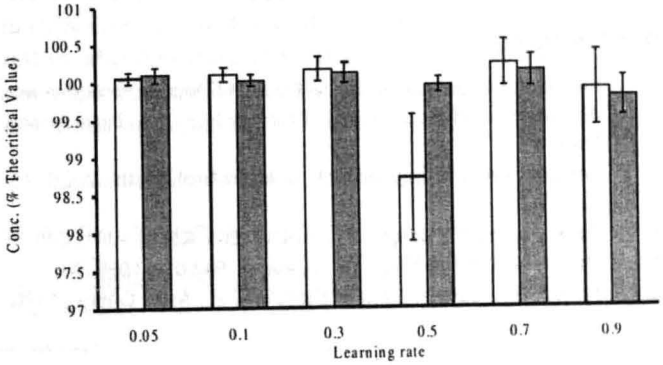

Fig. 5: Effect of learning rate on ANN performance.

Effect of learning rate on the accuracy of prediction of DIC (-□-) and PAR (-■-) concentrations by ANN with 16 input neurons, wavelength range 220 to 295 nm, 169 training pairs, sigmoid activation function, random initialization and 0.0001 error tolerance.

trained in 177 min and the trained ANN could predict the concentration with a mean of 100±0.33% at 99% CL with 0.1 SEM for DIC and 100±0.27% at 99% CL with 0.08 SEM for PAR. The study indicated that the neural network model could be a promising and powerful tool for the simultaneous estimation of substances in multi-component sample once the optimum ANN training parameters for the specific multi-drug formulation is established.

ACKNOWLEDGEMENTS

The authors thank the AICTE, New Delhi, for providing financial assistance under an R&D scheme to carry out this work.

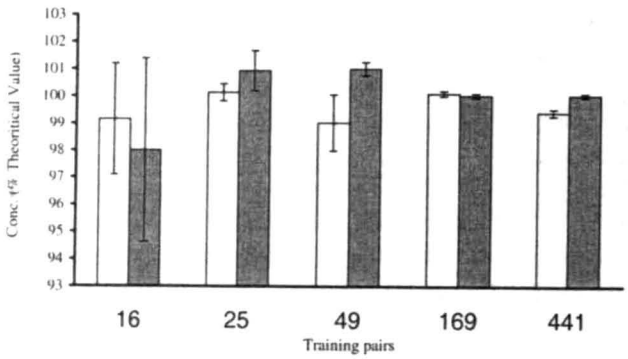

Fig. 6: Effect of number of training pairs on ANN performance.

Effect of error tolerance on the accuracy of prediction of DIC (-□-) and PAR (-■-) concentrations by ANN with 16 input neurons, wavelength range 220 to 295 nm, sigmoid activation function, random initialization 0.1 learning rate and 0.0001 error tolerance.

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