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Spectrophotometric Determination of Hexose and Pentose Amounts by Artificial Neural Network Calibration and Its Using in Wood Analysis

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Abstract

In this study, hexose (glucose) and pentose (xylose) in mixture solutions were substituted with anthrone, and their spectrophotometric absorbance values at 540 nm were recorded. MATLAB software was applied for data treatment as a multivariate calibration tool in the spectrophotometric procedure. The artificial neural network (ANN) trained by the back-propagation learning was used to model the complex relationship between the concentrations of hexose and pentose and the absorbance values of sugar mixture solutions. The optimized network predicted the hexose and pentose amounts in the mixture solutions. The ANN used can be proceed the data with an average relative error of less than 1.40%. Furthermore, the hexose and pentose amounts of pine wood sample were estimated by ANN and compared with gas chromatographic results of the same sample. The percent differences between predicted and gas chromatographic results were found as 6.62% for pentose and 1.44% for hexose, respectively.

Key words: hexose, pentose, wood, anthrone, artificial neural network (ANN)

Introduction

Wood consists of three major chemical components which are cellulose, polyose (hemicellulose) and lignin. Cellulose, before polyose, is the most abundant carbohydrate in wood. Efficient utilizations of the cellulose component of wood are to priority use the production of fibrous material for processing paper, rayon or cellulose derivates, while polyose is obtained as degraded and dissolved compounds in pulping liquors.[1] On the other hand, wood carbohydrates are abundant potential raw materials after conversation in to monosaccharides. They are hydrolysed to a variety of hexoses and pentoses, including glucose, mannose, galactose, xylose, arabinose and rhamnose offered to use the production of different chemicals.[1–7]

The anthrone method is currently a common colorimetric procedure for the analysis of the total sugar determination. This procedure is quick and reproducible technique. Anthrone produces a blue colour in reaction with hexoses and a yellow-green colour in reaction with pentoses.[8] In the previous studies, this method was generally used in the determination of the total sugar amount in the hexose solutions or the solutions rich in hexose. The colorimetric response is compared to a standard curve based on glucose.[9–17]

Artificial neural networks (ANNs) are information processing models inspired by structure and function of biological neurons. A description of the applications of ANNs to chemistry can be found in details elsewhere.[18–20] The applications of ANNs have been reviewed for calibration[21–24] and spectrophotometric researches.[25–31]

ANNs consist of neurons or nodes sorted into three different layers which are input, hidden and output layers. Neurons are interconnected in the neural net. These interconnections are computed with weights of neural connecting. Sigmoidal logistic function is often used as activation function in the neural network. The logistic function and the back propagation algorithm are used for weights optimization. The back propagation algorithm with the deltarule or other suitable method is called a learning method because weights are adapted to minimize the error between the desired target values and their calculated values by the neural network. By using of the test data sets, the weights have been determined to find the network accuracy in the prediction of external data sets. This cycle is repeated for different networks to determine an optimum network structure.[32–38]

In this study, it was used ANN with back propagation of error algorithm for modelling the complex relationship between colorimetric response of sugar mixture and concentrations of hexose and pentose. After total hydrolysis, the absorbance value of anthrone-substituted wood monosaccharides can be obtained spectrophotometrically. By using the absorbance value of wood sample, the hexose and pentose concentrations were tried to predict with ANN.

Experimental

Apparatus

In a Retsch SK 1 mill, the wood material was milled. Büchi Extraction System B-811 was used for the alcohol extractions of wood sample, P-Selecta Autoclave for acid hydrolysis and Perkin Elmer Lambda 20 UV/VIS Spectrometer for the absorbance value measurements.

Reagents

D(+) glucose, D(+) xylose, anthrone, cyclohexane, ethanol and sulfuric acid were purchased from Merck.

Procedure

Glucose (hexose) and xylose (pentose) were used as standard sugars. Each sugar solution was prepared by dissolving 50 mg of dried standard in 250 mL of distilled water. From these solutions, standard mixture solutions were prepared by using 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65 μg glucose concentrations, respectively and 5, 30, 40, 50 and 60 μg xylose concentrations, respectively. Each glucose concentration was mixed with xylose concentrations and diluted to 1 mL by adding distilled water. So it was obtained 50 standard mixture solutions for using in process. 2 mL anthrone reagent (200 mg anthrone suspended in 100 mL 96–98% H_2SO_4) were added to each standard mixture solution. The solutions were heated for 10 min in a boiling water bath. After cooling, the absorbance values of solutions were obtained at 540 nm.[11] Each blank-substracted absorbance value was found as average of 10 measurements, and recorded. These spectrophotometric data and concentrations of sugar standards were used to construct ANN models.

The used test wood (*Pinus sylvestris* L.) was ground to 40–100 mesh and extracted with 2:1 cyclohexane and ethanol followed by extraction with ethanol.

The sample size for acid hydrolysis was 200 mg extracted wood meal (on oven dry basis). The sample was primary hydrolyzed for 1 hour at 30 °C in 72% H_2SO_4, then diluted to 3% and secondary hydrolyzed for 1 hour at 120 °C in autoclave.[39] After filtering and washing the insoluble lignin (Klason lignin), the filtrate and washing water were combined and transferred to 1 L volumetric flask. 0.5 mL of 1 L solution was diluted to 1 mL by adding distilled water. The absorbance value of this 1 mL solution was measured at 540 nm after adding anthrone reagent, boiling and cooling procedures. The blank-substracted absorbance value was found as average of 10 measurements, and recorded to predict hexose and pentose amounts by ANN models.

Methodology

50 data pairs were split into a training set (randomly 35 data pairs), a test set (randomly 10 data pairs), and a validation set (5 data pairs), which were used to construct the neural network models. The input (absorbance values) and output (concentrations of hexose and pentose) data sets must be normalized into a range 0.1–0.9. For normalizing of data sets, the following expression was used:

$$X_N = 0.1 + \frac{0.8(X - X_{\min})}{(X_{\max} - X_{\min})}. \qquad (1)$$

where X_N is normalized value of the network input data or the network output data, X is original value of the data, and X_{max} and X_{min} are the maximum and the minimum original values of the data, respectively. The predictive ability of the different ANN models was assessed in terms of the root mean square (RMS) error values for the validation. RMS error values were obtained by

$$RMS = \sqrt{0.5N^{-1}\sum_{i=1}^{N}\left(X_i^{'} - X_i\right)^2}\ . \qquad (2)$$

where N is the number of testing data, $X_i^{'}$ the target value, and X_i is the output value produced by the network.

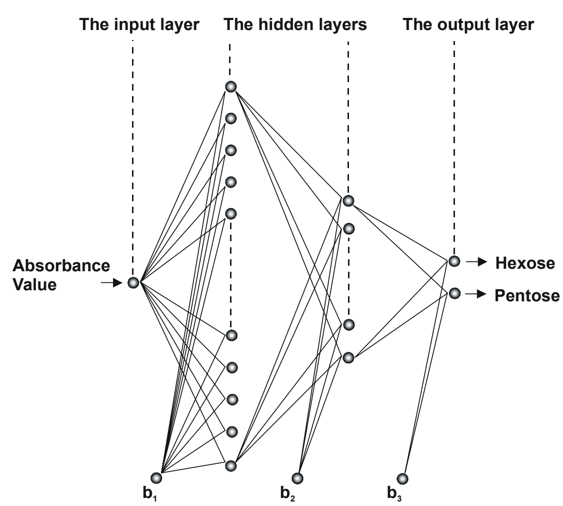

Figure 1. Network architecture used in the spectrophotometric method.

MATLAB software was used to construct ANN models which have sigmoidal logistic function with back propagation of error algorithm. For this neural network modelling an input layer, one or two hidden layers and an output layer were used. As seen from Figure 1, a neuron which is the absorbance value of standard mixture was used in the input layer, and two neurons which are the concentrations of hexose and pentose in the mixture solutions were used in the output layer. The number of hidden layer and hidden neurons were determined, and RMS error value was minimized to find a proper network structure for the validation step.

Results and discussion

The absorbance values of pentose and hexose standard mixtures which were used as input variables in the network architecture are shown in Figure 2. The absorbance values were varied between 0.137 and 0.620.

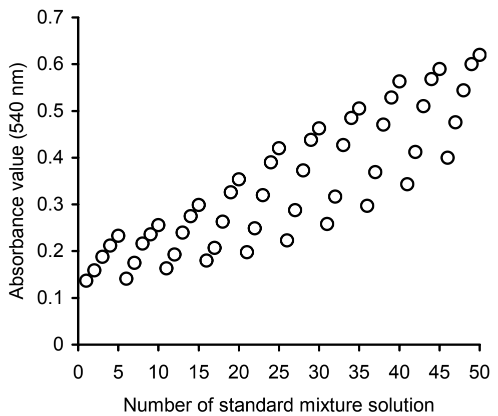

Figure 2. The spectrophotometric absorbance values of standard mixtures at 540 nm.

After training and testing, the results provided by the various neural network models, which have the logistic function, were assessed in term of RMS errors, and the number of the hidden layer and hidden neurons of the network was determined. Based on RMS errors given Table 1, NN5 model with a neuron in the input layer, 13 neurons in the first hidden layer, 6 neurons in the second hidden layer and 2 neurons in the output layer performed best on the testing data sets, and this **NN5 1-13-6-2** model was selected to predict the hexose and pentose concentrations.

Table 1. Comparison of the performances of the neural network models.

Model	RMS error			
	Hexose		Pentose	
	Training	Testing	Training	Testing
NN1 1-11-2	0.054534	0.119188	0.146838	0.231999
NN2 1-13-2	0.046942	0.114812	0.111697	0.194798
NN3 1-13-2-2	0.048504	0.096646	0.093057	0.177165
NN4 1-13-4-2	0.020846	0.039487	0.027243	0.027365
NN5 1-13-6-2	0.005957	0.004170	0.008238	0.005004
NN6 1-13-8-2	0.019040	0.010179	0.023003	0.025181
NN7 1-15-2	0.058488	0.109086	0.168581	0.242380

Regression plots of predicted values of hexose and pentose concentrations from NN5 model and the output (target) values are shown for the training data set in Figure 3 and for the testing data set in Figure 4. The NN5 model scored correlation R^2 values skill of 0.999, 0.9996, 0.996 and 0.998, respectively. The predicted results from the structured model approximated the actual results quite well.

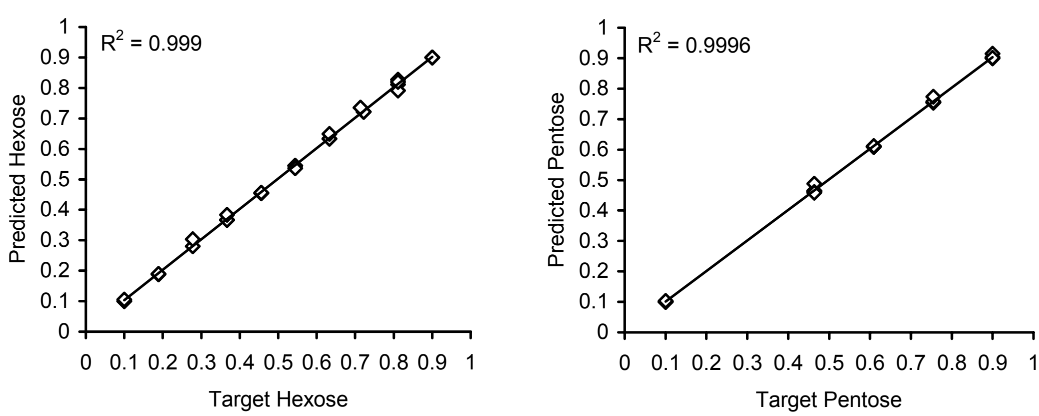

Figure 3. Comparison of predicted results from the NN5 model with target values from the actual values hexose and pentose for the training data set.

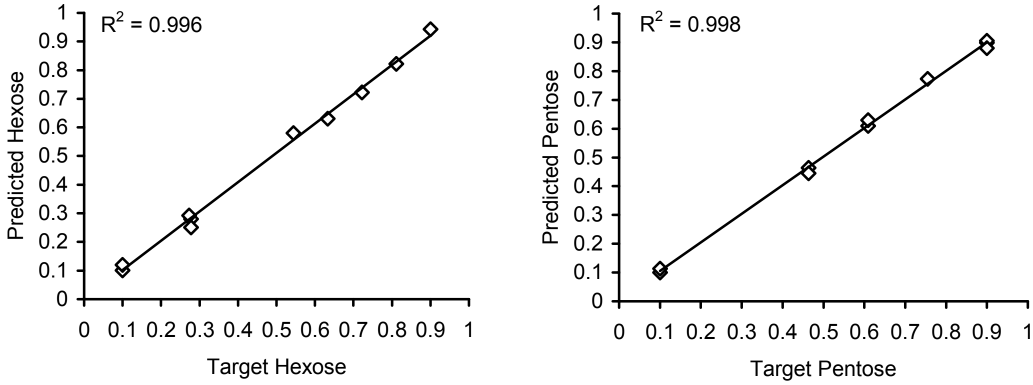

Figure 4. Comparison of predicted results from the NN5 model with target values from the actual values hexose and pentose for the testing data set.

Five standard mixture solutions were used to show validation of the model selected. Experimental results and estimated results from the model were listed in Table 2. As seen from the table, the error in the obtained estimation is small. The percent relative standard error of prediction is varied between –2.37 and 3.63. The low average relative error of prediction (<1.40%) proves that the network models have good predictive ability for application.

Table 2. Statistical parameters calculated for the prediction set using optimized ANN model.

	Sugar mixture					
	Hexose			Pentose		
	Actual (ppm)	Predicted (ppm)	RE %	Actual (ppm)	Predicted (ppm)	RE %
1	25	25.23	0.92	50	50.13	0.26
2	35	36.27	3.63	40	40.58	1.45
3	45	45.29	0.64	50	50.17	0.34
4	50	49.38	–1.24	30	29.29	–2.37
5	65	66.02	1.57	50	50.78	1.56

Furthermore, hexose and pentose amounts of pine wood (*Pinus sylvestris* L.) were estimated by ANN5 model and expressed as percent in oven dried extracted wood. The predicted amounts were 9.83% pentose and 60.97% hexose, respectively. In the same wood sample, 9.2% pentose and 60.1% hexose (based on oven dried extracted wood) were found by gas chromatographic method.[40] The percent differences between predicted and gas chromatographic results were calculated as 6.62% for pentose and 1.44% for hexose, respectively.

Conclusions

The neural network with a reasonable error have a good potential to predict hexose and pentose concentrations of the mixture solutions. The neural network modelling used in the spectrophotometric method can estimate the amount of hexose and pentose in unknown sample solutions. ANN also demonstrates low prediction errors (<1.40%) and high correlation R^2 values (0.999, 0.9996, 0.996 and 0.998, respectively) emphasized the high linear relationship between the predicted and the actual concentrations of hexose and pentose. The estimated results of pine wood by ANN are in good agreement with the results obtained by gas chromatographic method (Difference% for hexose 1.44 and for pentose 6.62).

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Povzetek

V pričujoči raziskavi smo mešanici heksoze (glukoza) in pentoze (ksiloza) v raztopini z dodatkom antrona določili absorbance pri 540 nm in uporabili MATLAB programsko opremo za multivariatno umeritev. Nevronske mreže s povratnim učenjem smo uporabili za modeliranje razmerja med koncentracijami heksoze in pentoze v mešanicah, tako da smo lahko napovedali koncentracijo posameznega saharida z relativno napako manj kot 1,4%. Nadalje smo s to metodo določili količine pentoz in heksoz v vzorcu borovega lesa in rezultate primerjali s kromatografsko analizo. Razlike so znašale v primeru pentoz 6,62% in v primeru heksoz 1,44%.