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Simultaneous Spectrophotometric Determination of Tartrazine, Sunset Yellow and Allura Red in Commercial Products by Artificial Neural Network Calibration

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The artificial neural network (ANN) trained by back-propagation learning was applied to process spectrophotometric data of mixed tartrazine, sunset yellow and allura red. By optimizing the network structure, accuracy was enhanced. The aim of this work is to propose the application of ANN methods to resolve mixtures of colorants in commercial products by using solid phase spectrophotometry. The proposed methods are rapid, easy to apply, inexpensive and suitable for analyzing the colorants in commercial products. The method takes advantage of sensitivity and selectivity.

Key Words: UV-Vis spectra, Simultaneous determination, Artificial neural network.

INTRODUCTION

At present, synthetic single colorants or mixtures of two or three colorants are frequently used as additives in foods or medicine and cosmetic products to obtain attractive colours, to correct the colour or for the identification of a product. The substances used as colorants are commonly synthetic products added in small amounts to food or drug products[1]. However, the use and the amount of these chemicals must be controlled because they can occasionally produce allergy, asthma and other health disorders in sensitized individuals[2]. Thus, in most of the countries they are strictly limited in use. Consequently, it is important to analyze their contents in those materials that are used by people.

Generally, spectrophotometry is used for determining these colorants and a prior separation is always involved, for there is a serious spectral overlap. The procedure of chemical separation is usually time consuming and often unsuccessful. An analysis that requires little or no separation is far more appealing. In order to avoid time-consuming clean-up procedures, attempts to resolve complex spectra by using instrumental approaches or various chemometric methods have been made. Derivative techniques[3] and multivariate statistical analysis[4, 5], such as multiple linear regression (MLR), principal component regression (PCR), partial least squares (PLS)[6, 7], have been successfully applied to the multicomponent analysis of mixtures by ultraviolet/visible molecular absorption spectrophotometry. Recently, derivative ratio spectrum method[8, 9] and branch-and-bound algorithm[10] have been successfully introduced for the determination of mixtures of colorants with three or more component combinations. Other graphical methods such as ratio spectra-zero crossing method[11] and double divisor-ratio spectra derivative method have been used for resolution of the ternary mixtures without requiring any separation procedures. To the best of our knowledge, no artificial neural network calibration of synthetic food colorant mixtures has been reported hitherto.

The aim of this work is to propose the application of ANN methods to resolve mixtures of colorants in commercial products by using solid phase spectrophotometry. The proposed methods are rapid, easy to apply, not expensive and suitable for analyzing colorants in commercial products. The method takes advantage of sensitivity and selectivity.

EXPERIMENTAL

All solvents and reagents were of analytical reagent grade unless otherwise indicated. Tartrazine (STA), sunset yellow (SSU) and allura red (SAL) were supplied by Aldrich Products (Milwaukee, USA). Stock solutions of STA, SSU and SAL, 25 mg/100 mL of each, were prepared in 0.1 M HCl. Absorbance measurements were carried out by using a Perkin-Elmer Lambda 20 double beam UV-Vis spectrophotometer interfaced to an IBM SX-486 microcomputer for the spectral acquisition provided with a UV-Win Lab software and subsequent manipulation of the experimental data. The absorbance measurements were carried out in two matching quartz 1.0 cm cells with a 1 mm path length.

Absorbance measurements: The absorption spectra were recorded between 200 and 620 nm with an interval of 0.1 nm between each two points and were contrasted with the corresponding blanks. The spectra were giving 4200 experimental points for each spectrum. The calibration matrix was prepared from 18 solutions containing mixtures of three components in different ratios and optimized and calculated by using ANN calibration both to analyze the spectra obtained and to calculate the concentration of the analytes in the real samples.

Procedures

An aliquot of sample containing between 2 and 30 μg of STA, SSU and SAL (alone or in mixtures) was placed in a 25 mL volumetric flask and 0.1 M HCl was added. The mixture was shaken for 10 min and packed in a 1 mm cell. Blanks were prepared in the same way as described for the standards and contained all the reagents except the colorants.

Procedure for real samples

For this purpose, an amount equivalent to 2.0 g samples (five times) in 45 g soft drink powder (commercial product, Tang®, Marsa Kraft Jacobs Suchard Sabanci Food Ind.) was transferred to 25 mL calibrated flask and dissolved in 0.1 M HCl mechanically. The solution was centrifuged for 20 min at 5000 rpm and the supernatant was transferred to a 25 mL volumetric flask. The volume was completed with the same solvent.

Methodology

A feed-forward ANN model with four layers of nodes was constructed as in Fig. 1.

The logistic function was used as the activation function in a neural network. The training and testing data sets must be normalized into a range 0.1–0.9[12]. The input and the output data sets were normalized by using the following equation:

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

where X_N is the normalized value of a variable (the network input or the network output), X is the original value of a variable, and X_{max} and X_{min} are maximum and minimum original values of the variables, respectively. In order to produce sufficient data for training and testing of the model shown in Fig. 1. Eighteen different standard solutions were prepared using different colorant concentrations and each standard solution was subjected to spectrophotometric determination. Randomly chosen 72 data pairs from these 180 data pairs were used in the training of the neural network and the rest of the data were used in the testing. The root mean square error values were calculated from the following equation to prove quantitatively the accuracy of the testing results of neural network models:

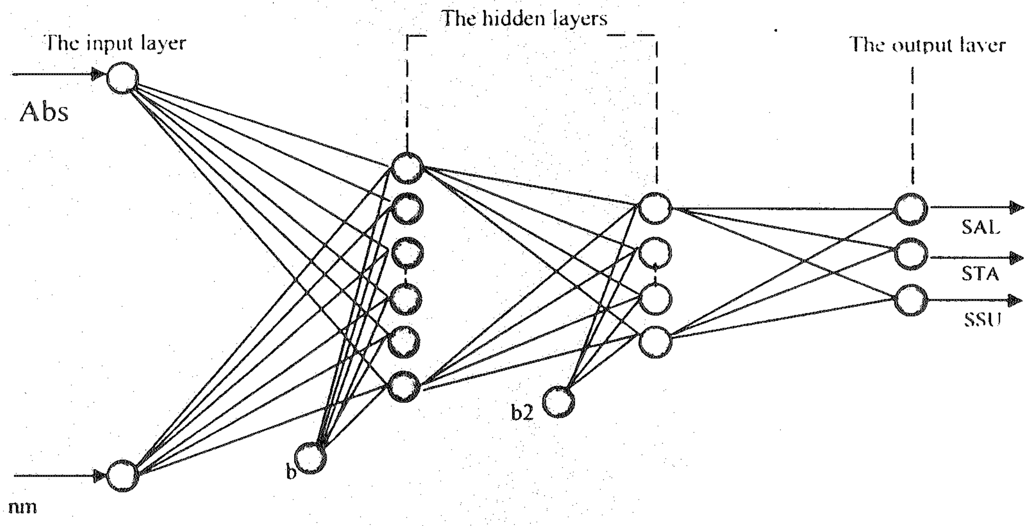

Fig. 1. Network architecture used in the spectrophotometric determination

$$RMS = \sqrt{0.5\, N^{-1} \sum_{i=1}^{N} (X_i' - X_1)^2} \qquad (2)$$

where N is the number of testing data and X_i' is target value.

RESULTS AND DISCUSSION

The colorants used in this study are chemically related compounds. Fig. 2, 3 and 4 show the absorbance-wavelength curves of these colorants. It is obvious that spectra of these three colorants are overlapped seriously.

To obtain the best network performance, the optimal network architecture and parameters must be chosen. Studies of the network structure include the selection of the number of layers and number of nodes in each layer. The number of layers used for this neural network modelling was four, *i.e.*, an input layer, one or two hidden layers and an output layer. As can be seen from Fig. 1, two neurons were used in the input layer, which were the absorbance and wavelength (nm), and those of the hidden layer were optimized for each colorant and mixture solutions. The absorbance and wavelength of the solution were considered as independent variables of the spectrophotometric method. Therefore, these variables were used as input variables in the network architecture. In Table-1, the concentration of standard solutions is represented (or output data of the network).

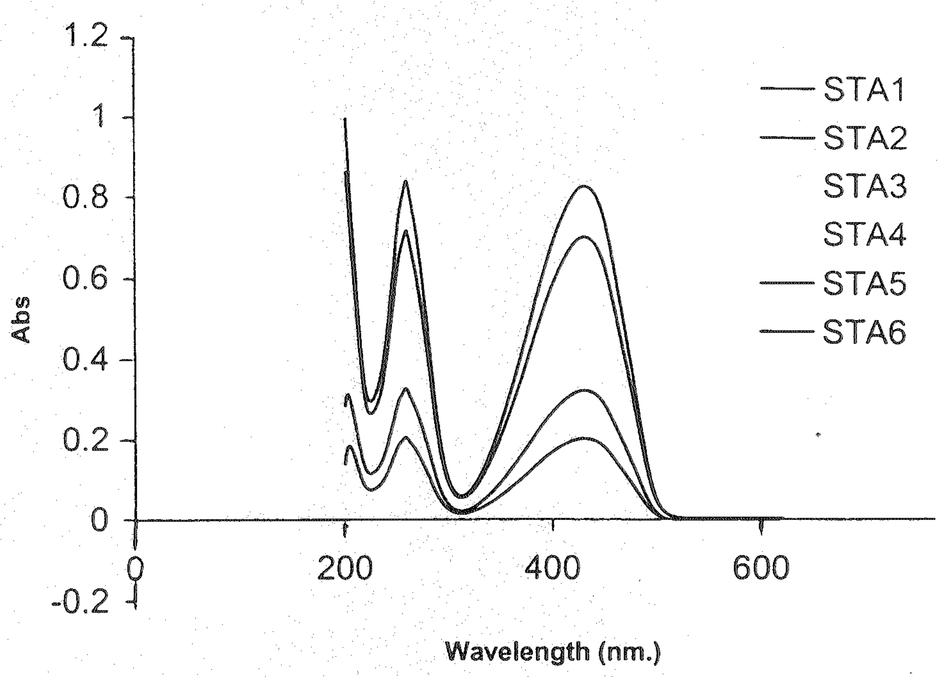

Fig. 2. Absorbance-wavelength curves for STA

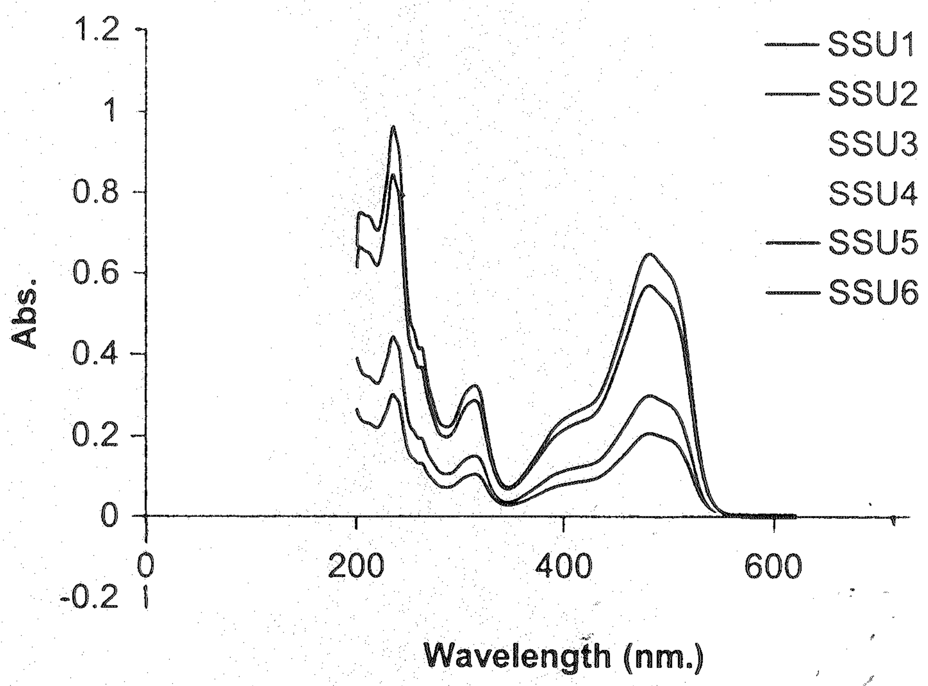

Fig. 3. Absorbance-wavelength curves for SSU

Generally, the absorbance detection was the first choice in the spectrophotometric analysis of food colorants owing to its simplicity and reliability. The colorants themselves were often detected in the visible wavelength range and the intermediates as well as other organic impurities in the ultraviolet range. In this study, the experimental results obtained from a spectrophotometer showed that the absorban-

TABLE-1
RECOVERY TABLE OF MIXTURES CONTAINING THREE COLORANTS

Sample number	Concentration (μg/mL)			Sample number	Concentration (μg/mL)		
	Sunset yellow	Tartrazine	Allura red		Sunset yellow	Tartrazine	Allura red
1	4	18	6	10	12	18	6
2	6	18	6	11	12	22	6
3	8	18	6	12	12	26	6
4	10	18	6	13	12	18	4
5	12	18	6	14	12	18	6
6	14	18	6	15	12	18	8
7	12	6	6	16	12	18	10
8	12	10	6	17	12	18	12
9	12	14	6	18	12	18	14

ces of all colorants in the ultraviolet range were not higher than those in the visible range and the background absorbance of the eluent in ultraviolet range was very high. The maximum absorption wavelength and the optimal detection wavelength of each colorant were in the visible range. Because of the significant differences between the maximum absorption wavelengths, a wavelength switching technique should be a better solution for improving the detection limits of all colorants.

The various neural network models, which have the logistic function, were trained and tested. In this step, the number of hidden layer units of the network was determined by performance evaluating of the network models defined. The NN-2-24-36-3 model, which performs best on the testing data set, was selected as neural network model to predict the colorant concentrations.

The plot of predicted concentration of colorants *vs.* actual concentrations is shown in Fig. 5. The fit of data to a straight line (with a $r^2 = 0.9956$) confirms the excellent predictive ability of the networks used in this study. Meanwhile, the correlation coefficients of such plots for individual colorants (*i.e.*, 0.9927, 0.9963 and 0.9967 for SSU, STA and SAL, respectively) emphasize the high linear relationship between the predicted and the actual concentrations.

Experimental results and estimated results from the model are given in Table-2.

TABLE-2
STATISTICAL PARAMETERS CALCULATED FOR THE PREDICTION SET USING OPTIMIZED NETWORK MODELS

Sample Number	Colorant mixture composition								
	Sunset yellow			Tartrazine			Allura red		
	Actual (μg/mL)	Predicted (μg/mL)	RE (%)	Actual (μg/mL)	Predicted (μg/mL)	RE (%)	Actual (μg/mL)	Predicted (μg/mL)	RE (%)
1	0.2857	0.28566	–0.0140	0.2307	0.23062	–0.0347	0.2857	0.28571	0.0035
2	0.4285	0.42855	0.0117	0.3846	0.38458	–0.0052	0.4285	0.42851	0.0023
3	0.5714	0.57177	0.0647	0.5384	0.53846	0.0111	0.5714	0.57133	–0.0123
4	0.7142	0.71417	–0.0042	0.6923	0.69222	–0.0116	0.7142	0.71425	0.0070
5	0.8514	0.85152	0.0141	0.8461	0.84617	0.0083	0.8514	0.85146	0.0070
6	1.0000	0.99928	–0.0720	1.0000	0.99878	–0.1220	1.0000	0.99962	–0.0380

RE: Relative error.

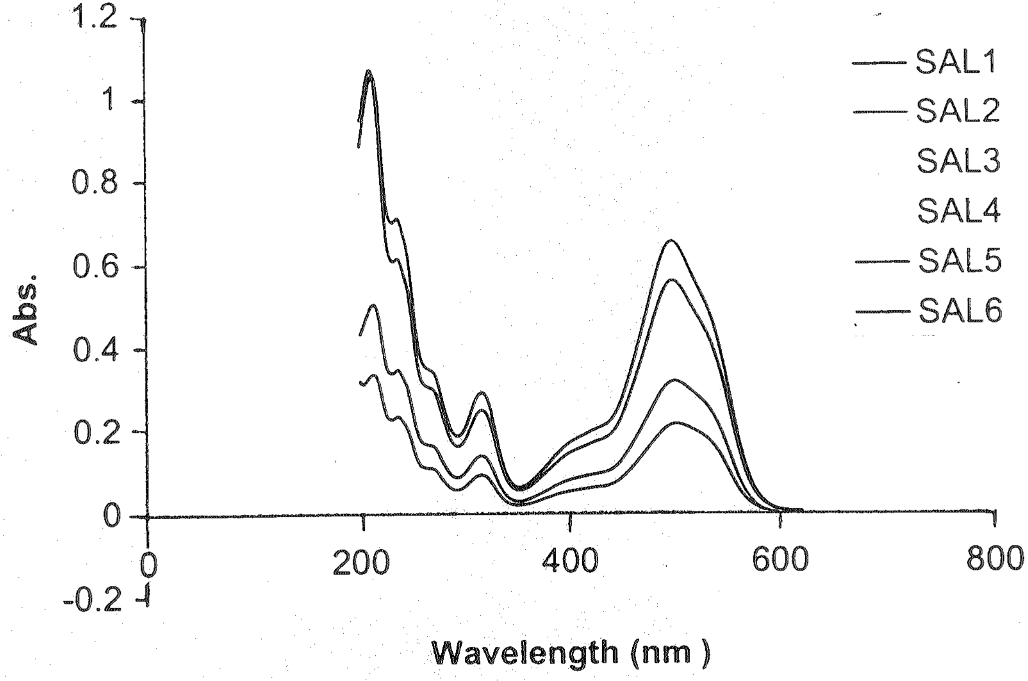

Fig. 4. Absorbance-wavelength curves for STA

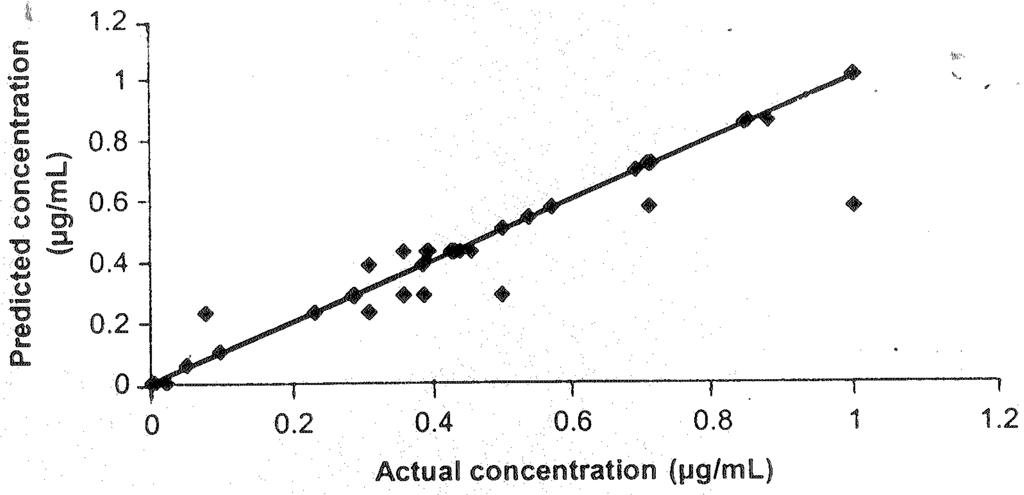

Fig. 5. Linear plot of predicted concentration of colorants by ANN *vs.* actual concentrations

As can be seen from the table, the error in the obtained estimation is at negligible level. The per cent relative standard error of prediction is varied between –0.072 and 0.0647. The low average relative error of prediction indicates that the networks used can properly process the spectrophotometric data and the model the complex relationship between the concentration of colorants in the mixture and wavelength at different absorbances. In the ANN approaches, another important parameter is the standard error of prediction (SEP). The SEP values and their statistical values computed are given in Table-3. It can be seen from this table that the mean recovery values are good evidence of the validity of the method.

TABLE-3
RECOVERY DATA OBTAINED BY APPLYING ELABORATED ANN APPROACHES TO SYNTHETIC MIXTURES

Added µg/mL			Recovery (%)		
Sunset yellow	Tartrazine	Allura red	Sunset yellow	Tartrazine	Allura red
4	18	6	99.98	91.54	97.99
6	18	6	100.01	100.10	105.20
8	18	6	100.06	87.90	106.17
10	18	6	99.99	96.66	102.67
12	18	6	100.01	98.17	90.49
14	18	6	99.92	100.02	98.67
12	6	6	100.19	99.98	97.84
12	10	6	102.32	99.99	92.07
12	14	6	98.32	100.01	101.65
12	18	6	99.23	99.98	99.23
12	22	6	101.19	100.00	100.19
12	26	6	100.71	99.87	96.98
12	18	4	101.89	99.97	100.00
12	18	6	98.47	97.73	100.00
12	18	8	97.26	106.98	99.98
12	18	10	95.67	99.39	100.01
12	18	12	100.71	99.89	100.01
12	18	14	99.94	99.29	99.96
		Average	99.77	98.75	99.39
		SD	1.58	3.90	3.77
		RSD	1.58	3.95	3.80

SD: Standard deviation; RSD: Relative standard deviation

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