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Evaluation of an optical phenolic biosensor signal employing artificial neural networks

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ABSTRACT

This paper presents artificial neural network (ANN)-based evaluation in signal processing of an optical phenolic biosensor. The biosensor was developed based on stacked immobilization of 3-methyl-2-benzothiazolinone hydrazone (MBTH) in hybrid Nafion/sol–gel silicate and tyrosinase in chitosan. The biosensor signal was simulated employing a feed-forward neural network with three layers and trained using back-propagation (BP) algorithm. Spectra generated from an optical phenolic biosensor at selected wavelengths were used as input data for ANN. The network architecture of 5 inputs neurons, 21 hidden neurons and 1 output neuron was found suitable for this application. The results show very good agreement between phenol concentration values obtained by using the developed biosensor and those predicted by ANN.

1. Introduction

The use of biosensors have become increasingly important as a system or analytical tools in certain areas like environmental monitoring [1,2], medical applications [3] and bioprocess control [4]. They offer rapid, sensitive and accurate analysis, faster response, simple technique and lower cost. Biosensors have been growing through many technical improvement and advances, and have attained a very high level of sophistication in many aspects. One of the areas is the application of powerful chemometric tools in processing of biosensor signals like artificial neural network (ANN) [5]. ANN could be used for modelling and calibration of complex analytical signals [6]. In the cases where sensors show non-linear response and operate in a short linear region [7–9], ANN plays an important role in non-linear adaptive filtering or processing of the sensor response in order to increase the linearity and produce stable measurement. Adaptive means that the system parameters are changed during the training process. The training mechanism involves collection of pre-measured data and responses for each of the input and output neurons employed and the execution of a well-designed training algorithm [9]. Once the training process is completed, the ANN parameters are fixed and it is able to provide an accurate prediction during recall process.

ANN is a computing system based on adaptation of human brain, which is made up of simple number and highly parallel interconnected processing elements, namely neurons [10]. ANN, like human being, learns by example. ANN is configured for specific applications, such as pattern recognition, data classification, signal interpretation or prediction through a learning process. Learning in biological nervous systems involves adjustments to the synaptic connections that exist between the neurones. The similar concepts also happen in ANN as well.

Recently, several studies on the application of ANN for processing of sensor signals have been published [2–4,11,12]. Torrecilla et al. [11] presented application of ANN for determination of concentration of phenolic compounds based on an integrated ANN/laccase biosensor in olive oil mill wastewater. Gholmieh et al. [12] demonstrated the detection and classification of neurotoxins using a tissue-based biosensor in combination with ANN for pharmacological application.

This paper describes the development of an optical biosensor based on stacked immobilization of 3-methyl-2-benzothiazolinone hydrazone (MBTH) in hybrid Nafion/sol–gel silicate and tyrosinase in chitosan for the determination of phenolic compounds. Several papers have reported the use of a sol–gel matrix for biosensor development. However, the cracking of the sol–gel matrix with time is still a problem [1,13,14]. To solve the latter problem it was hybridized with a polymer such as Nafion. Nafion is a perfluorinated polymer that contains a hydrophobic fluorocarbon backbone, and the hydrophilic cation-exchange site thus exhibits moderate hydrophobic properties [13]. It was mixed with sol–gel silicate to

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form an organic–inorganic hybrid material to overcome the brittleness and shrinkage of the pure sol–gel-derived silicate. Other advantages of this material are to provide moderate hydrophobic environment for MBTH immobilization, to retain the hydrophilic MBTH reagent and prevent its leaching from the biosensor film, and to permit permeation of the analyte into the material structure where the recognition can occur [14].

The application of ANN technique for evaluation of the signals generated by the developed optical biosensor was explored in this work. A feed-forward neural network was used to simulate this process employing a single hidden layer combined with back-propagation algorithm in the network training. It was the aim of the study to extend the limited measuring range of the developed optical biosensor to a useful and wider working region.

2. Experimental

2.1. Reagents

Tyrosinase from mushroom (EC 1.14.18.1) and phenol were purchased from Sigma. Nafion (5% solution in a mixture of alcohol) was supplied by Aldrich. Hydrochloric acid (37%) and acetic acid glacial were obtained from R & M Chemicals and Ajax Chemicals, whereas MBTH was purchased from Merk. Tetraethyl orthosilicate (TEOS) was acquired from Fluka. Chitosan was supplied by Chito-Chem (Malaysia) Sdn. Bhd. All chemicals were used without any further purification.

2.2. Preparation of stock solution

The stock sol–gel solution was prepared by vigorously mixing 1000 μL of TEOS, 200 μL of distilled water and 10 μL of 0.1 M HCl (the molar ratio of TEOS:H_2O:HCl was 4500:11,000:1) in a glass vial. The sol–gel solution was stirred for 24 h. The sol–gel silicate solution was mixed homogeneously with a Nafion solution at a volume ratio of 1:1 (v/v) and kept at room temperature (25 °C) overnight. Then a small portion of MBTH solution (100 mM) was mixed to hybrid the Nafion/sol–gel silicate mixture at a volume ratio of 0.3:1 (v/v), and subsequently it was stirred until a homogeneous solution was obtained.

A chitosan solution (2%, w/v) was prepared by dissolving 2 g of chitosan powder in 100 mL of acetic acid (1%, v/v). The viscous chitosan solution was stirred overnight at room temperature. A homogeneous stock solution of tyrosinase/chitosan mixture was prepared by mixing the chitosan solution and a tyrosinase solution (150 mg/mL) at a volume ratio of 15.7:1.0 (v/v) [14] in an Eppendorf tube of 1 mL. The mixture was stirred gently for 15 min. This stock solution was freshly prepared before fabrication of the optical film.

2.3. Fabrication of biosensor

Ten microliters of the hybrid Nafion/sol–gel silicate–MBTH mixture was initially deposited onto a glass slide (25 mm × 9 mm) and smeared in an area of 9 mm × 10 mm. Then it was spun at 3000 rpm for 5 s. The glass slide with a hybrid Nafion/sol–gel silicate–MBTH film was kept in an air tight bottle at 4 °C overnight for drying purpose. A volume of 10 μL of the stock tyrosinase/chitosan mixture was then pipetted onto the dried hybrid Nafion/sol–gel silicate–MBTH film on a glass slide and coated gently over an area of 9 mm × 10 mm. Then it was spun at 2000 rpm for 3 s and dried at 4 °C overnight.

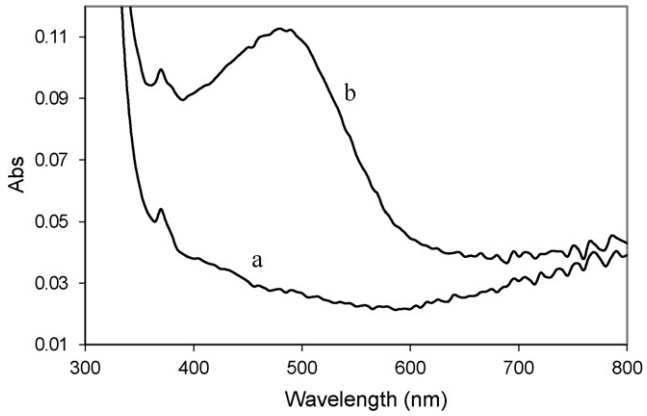

Fig. 1. The absorption spectra of the biosensor derived by stacked immobilization of MBTH in hybrid Nafion/sol–gel silicate and tyrosinase in chitosan in the (a) absence and (b) presence of 9.4 mg/L phenol.

2.4. Neural network architecture and optimization

A feed-forward neural network with back-propagation training algorithm was employed for the treatment of data. The input layer consists of five neurons corresponding to the contours of the absorbance intensity of a quinone–MBTH adduct measured at 350, 440, 490, 560 and 610 nm. The output layer consists of a single neuron of value corresponding to variable phenol concentration values. Networks having up to 23 neurons in the hidden layer have also been considered.

The ANN training and data treatment were conducted using a Matlab program [15] with an Intel Pentium 4 processor having 512 MB of RAM. A sigmoid transfer function was employed in the hidden and output layers. The network training was optimized with respect to the number of hidden neurons and epochs (the maximum number of cycles to train). The latter process involved cycling through the training data until the minimum error between the expected value and the predicted value was reached. Assessing of the network learning accomplishment was estimated from the sum-squared error (SSE) value which was usually employed to represent the difference, according to the following equation:

$$\mathrm{SSE} = \frac{1}{2}\sum_{i}(Y_i - Y_i')^2 \quad (1)$$

where Y_i is the expected value and Y_i' is the predicted value, which is calculated from the output produced by the ANN.

3. Results and discussions

Fig. 1 illustrates the spectra of the disposable biosensor derived by stacked immobilization of tyrosinase in chitosan and MBTH in hybrid Nafion/sol–gel silicate in the absence and presence of phenol. As shown, no signal was obtained in the absence of phenol. However, in the presence of phenol an optimum response of the biosensor was observed at a wavelength of 490 nm, indicating formation of a quinone–MBTH adduct. Similar finding was also reported previously [16,17], where a red color adduct was observed in the sensor reaction. Fig. 2 shows the response of the biosensor towards different phenol concentrations in the range of 0.5–20.0 mg/L at wavelength contours of 350, 440, 490, 560 and 610 nm. The plot of absorbance versus concentration of phenol (Fig. 2) displayed the non-linear characteristic of the biosensor response above the concentration of 6.0 mg/L. Based on the work previously reported [8], these types of data are suitable for non-linear modelling using ANN.