Extending the Range of an Optical Vanadium(V) Sensor Based on Immobilized Fatty Hydroxamic Acid in Poly (Methyl Methacrylate) Using Artificial Neural Network

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ABSTRACT
An artificial neural network (ANN) was applied for the determination of V(V) based on immobilized fatty hydroxamic acid (FHA) in poly(methyl methacrylate) (PMMA). Spectra obtained from the V(V)-FHA complex at single wavelengths was used as the input data for the ANN. The V(V)-FHA complex shows a limited linear dynamic range of V(V) concentration of 10 - 100 mg/L. After training with ANN, the linear dynamic range was extended with low calibration error. A three layer feed forward neural network using back-propagation (BP) algorithm was employed in this study. The input layer consisted of single neurons, 30 neurons in hidden a layer and one output neuron was found appropriate for the multivariate calibration used. The network were trained up to 10 000 epochs with 0.003 % learning rate. This reagent also provided a good analytical performance with reproducibility characters of the method yielding relative standard deviation (RSD) of 9.29% and 7.09% for V(V) at concentrations of 50 mg/L and 200 mg/L, respectively. The limit of detection of the method was 8.4 mg/L.

Keywords: Artificial neural network (ANN), V(V), fatty hydroxamic acid (FHA), poly(methyl methacrylate) (PMMA)

INTRODUCTION
Optical sensors have become major analytical tools in monitoring the nature of chemical in the environment. Optical sensors, often called “optodes”, are a particular type of chemical sensor where spectroscopic measurements associated with chemical reactions are carried out (Guell et al., 2007). Optical sensor based on the use of uv-visible spectrophotometry for the determination of V(V) was developed and PMMA membrane was applied as supporting material in this study. FHA was used as a new reagent for the determination of V(V) and showed good properties in our preliminary study using a manual batch method (Isha et al., 2003).

The FHA was synthesized by reacting hydroxylamine with refined, bleached deodorized (RBD) palm kernel olein (liquid phase from the fractionation of palm kernel oil) using lipase as biocatalyst. FHA is produced with glycerol as a bi-product (Suhendra, 2002). Fig. 1 shows the preparation reaction for FHA. The transfer of acyl group from a donor ester to hydroxylamine (aminolysis) was catalyzed preferentially by the reaction of free fatty
acids. The exact structure and the molecular weight of FHA have not yet been determined. The suggested complex formation structure of V(V)-FHA complex is shown in Fig. 2. FHA is a white colour solid and colourless when in liquid form. FHA is slightly soluble in alcohol but not soluble in water.

where R is a mixture of following fatty acid chain: caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, oleic acid and linoleic acid.

**Fig. 1: Preparation reaction for FHA**

**Fig. 2: Structure of V(V)-FHA complex**

The need for V(V) analysis in environmental analysis has increased after a report on the different biological roles of ionic forms of this species in plants, animals and humans. Human exposure to vanadium has severe effects on cell growth, cardiac muscle, diuretic kidney function (Gavasov, 2000) and symptoms such as nervous depression, coughing, vomiting, anemia and increased risk of lung cancer, that are sometimes fatal (Ahmad and Banoo, 1999). The neurotoxicity of vanadium can cause somnolence, convulsions, respiratory failure and gastrointestinal irritation with diarrhea (Faulkner-Hudson, 1964).

PMMA membrane immobilized FHA determine the V(V) in limited linear dynamic range. Therefore, a good approach must be taken to extend the response range of this optical sensor. ANN was found to be a suitable program to solve this problem. Taib et al. (1996) first introduced the use of ANN as the mechanism to modelling complex non-linear data, applications of ANN in optical fibre chemical sensor technology. Generally, ANN is a system loosely modelled on the human brain. It represents an important paradigm for classifying patterns or approximating complex non-linear process dynamics. These properties clearly indicate that neural network exhibit some intelligent behaviour, and are good candidate models for non-linear processes, for which no perfect mathematical model is available (Denai et al., 2007). It is an attempt to simulate within specialized hardware, the multiple layers of simple processing elements called neurons. Each neuron is linked to certain of its neighbours with varying coefficients of connectivity that represent the strengths of these connections (Gonzalez and Dankel, 1993; Zahedi, 1993; Simon, 1994). ANN has to be trained. This means that, given a set of input-output
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patterns (called the training set), the connection weights of the neural network are adjusted in order to approximate the input-output patterns provided in the training set according to some predefined criterion. After training, the neural network can be used to predict a new output pattern, based on the input pattern only. The adaptation law that allows adjusting the connection weights is called the learning algorithm (Denai et al., 2007).

The aim of this study is based on the application of ANN to extend the useful linear range in the determination of V(V) ion based on immobilized FHA in PMMA. ANN with feed-forward network having a single hidden layer and the back-propagation algorithm was applied in this work.

**EXPERIMENTAL**

**Reagent**

All chemicals used were of analytical grade and deionized water was used throughout for solution preparation. A stock solution (5.0 x 103 mg/L) of V(V) was prepared by dissolving 0.5 g of V2O5 (BDH) in 100 mL of 1.0 M HCl (Merck). Working standard solution of V(V) were prepared by appropriate dilution of the stock solution before use.

**Synthesis of FHA**

Hydroxylamine hydrochloride (Fischer), sodium hydroxide (J.T Baker) and crude palm kernel olein (Southern Edible Oil) in hexane (J.T. Baker) were reacted in the presence of Lipozyme (Novo Nordisk). The lypozyme used were able to catalyze hydroxylaminolysis reaction which shows the highest activity. This is probably because lipozyme is an immobilized lipase, which has more storage stability and more active lipase than native and modified lipases, and its presence at the interface of the system with others at the bottom of the water phases evokes the contact of all the components in the lypozyme system better than others. The reaction was carried out in a sealed glass flask in water shaker bath with continuous shaking. The Lipozyme was separated by a filter paper and the yield was dried. The products were purified by crystallization in hexane and dried.

**Reagent Immobilization**

The doped PMMA membrane was prepared by adding 0.7006 g of dry PMMA powder and 0.0405 g of FHA into 10 mL of tetrahydrofuran. Then 210 _L of tributyl phosphate was added into the mixture. The solution was mixed thoroughly and poured into a petri dish with a diameter of 10 cm. The mixture was left to dry overnight to enable a smooth and even membrane to form. The membrane was cut into1.0 cm x 4.0 cm size.

**Procedure**

The membrane was placed vertically inside a plastic cuvette. The absorption spectra of PMMA immobilized FHA alone and the complex formation between PMMA immobilized FHA and 100 mg/L was recorded at wavelength 350 to 700 nm. The absorbance was measured five minutes after placement of the membrane in the V(V) solution.

The dynamic range was studied by placing the PMMA membrane in different concentrations of V(V) solution, i.e. 1 - 110 mg/L. The absorbance was measured at a wavelength of 495 nm.

The reproducibility was studied at V(V) concentration of 50 ppm and 200 ppm. A total of ten different batches of similarly prepared membranes were immersed in the
same concentration of analyte solution. In this study, two different concentrations of V(V) solution were used, i.e. 10 mg/L and 200 mg/L. The absorbance was measured and the relative standard deviation in the measurement was calculated.

**INSTRUMENTATION**

Spectral measurements were made with an ultraviolet-visible spectrophotometer (Varian-Cary Win UV 100). For each concentration, the spectrum was scanned at wavelengths of 350 - 750 nm. A total of 20 spectral readings were obtained. Five of these spectra (V(V) concentrations of 31, 39, 46, 52 and 108 mg/L) were used for testing the trained network whilst the remaining spectra (10, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105 and 110 mg/L) were used for training the network.

**DATA TREATMENT AND ANALYSIS**

A feed-forward ANN having a single hidden neuron layer with back-propagation (BP) training algorithm was employed for treatment of the data. The input layer consists of single neurons, which represent the absorbance intensities measured at one wavelength from each spectrum. The output layer consists of a single neuron which represents the concentration value of V(V). A network having up to 40 neurons in hidden layer, was considered in this study.

The network training and data treatment were realized by using Matlab program (Matlab, 2004) under an Intel Celeron processor having 256 MB of RAM. The training and optimization process carried out in this study is shown in Table 1. The network was trained up to 10 000 epochs and the progress of the sum-squared error (SSE) between the calculated and the measured output was recorded. Finally, a new set of input data was introduced to the networks to check for prediction capability and precision.

<table>
<thead>
<tr>
<th>Specific Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum number of epochs to train</td>
<td>10 000</td>
</tr>
<tr>
<td>Sum-squared error (SSE) goal</td>
<td>0.02</td>
</tr>
<tr>
<td>Learning rate</td>
<td>0.003</td>
</tr>
<tr>
<td>Frequency of progress displays (in epochs)</td>
<td>500</td>
</tr>
</tbody>
</table>

The preference of the best network was based on several tests using the trained network that incorporates the inspection for training data fitting errors and prediction test of errors. The selected network was then applied for computer generated application where new measurements were taken, processed and converted to concentration values employed by the Matlab program simulation.

**RESULTS**

*Spectral Studies*

*Fig. 3* shows the absorbance spectra of the immobilized FHA pre and post reaction with V(V). The formation of the complex causes an increase in absorbance due to a change
in color of the membrane from colorless to dark purple. The maximum absorbance
difference of the two absorbance spectra was observed at 495 nm and this wavelength was
therefore used for further measurements.

THE DYNAMIC RANGE OF THE V(V) CONCENTRATION
The typical analytical curve of the sensor response as a function of V(V) concentration
is shown in Fig. 4. It shows that the sensing material produced a linear response when
the V(V) concentration is within the range of 10 - 100 ppm. The limit of detection was
calculated to be 8.4 ppm. According to IUPAC definition, the limit of detection has
defined as the concentration that produces a signal that exceeds the signal observed from
a blank by an amount equal to three times the standard deviation for the measurement
on the blank.

Fig. 3: Absorbance spectra of PMMA immobilized FHA before (A)
and after (B) reaction with 100 ppm V(V)

Fig. 4: The response curve of the PMMA immobilized FHA
towards different concentrations of V(V)
Multivariate Calibration Using ANN

Data obtained from uv-visible spectrophotometer were used as input to the ANN. Single wavelength point (459 nm) from each spectrum was chosen to represent the input data for the ANN to avoid several problems during network training periods (Garg and Bozink, 1972; Bos et al., 1993). The points selected, were due to their significant variations in the sensor signal.

Fifteen spectra were used for the training of the ANN. Network optimization was performed by changing the number of neurons in the hidden layer, the number of cycles during training and the percentage of learning rate. Table 2 shows the SSE values of the network with 5, 10, 15, 20, 25, 30, 35 and 40 neurons in hidden layer after completing the 10 000 epochs.

<table>
<thead>
<tr>
<th>Number of neuron in hidden layer</th>
<th>Sum-square error (SSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>194.5040000</td>
</tr>
<tr>
<td>10</td>
<td>56.1151000</td>
</tr>
<tr>
<td>15</td>
<td>128.5680000</td>
</tr>
<tr>
<td>20</td>
<td>3.9953800</td>
</tr>
<tr>
<td>25</td>
<td>0.5797560</td>
</tr>
<tr>
<td>30</td>
<td>0.0199902</td>
</tr>
<tr>
<td>35</td>
<td>0.0199961</td>
</tr>
<tr>
<td>40</td>
<td>0.0199976</td>
</tr>
</tbody>
</table>

For the network with five neurons in hidden layer, the convergence of SSE was observed to be very slow. The fastest convergence of SSE was achieved using 30 neurons in the hidden layer. The number of hidden neurons when arranged in declining SSE order was 5, 15, 10, 20, 25, 40, 35 and 30. Network trained with 10 000 epochs were suitable to be used in predicting the response of the concentration of V(V) since it showed a low SSE value. Zupan and Gasteiger (1991) reported that, ANN training by using much higher number of epochs usually caused problems such as over training and over fitting problems. Five calibration spectra (31, 39, 46, 52 and 108 mg/L) were employed to establish their prediction capability. The trained networks with different number of hidden neurons were present to improve the process in choosing the best network’s architecture (Bos et al., 1993; Taib and Narayanaswamy, 1997).

Different values of learning rate (0.0070 - 0.0001) from the networks consists 30 neurons in hidden layer after observation. As shown in Table 3, a learning rate of 0.0030 gave the lowest SSE value followed by 0.005, 0.0010, 0.0005, 0.0003, 0.0070 and 0.0001.

Table 4 shows the predicted concentration values against the expected concentration values measured using a uv-visible spectrophotometer. As shown in Table 4, the network with 20, 25 and 30 neurons in the hidden layer produced good predictions with average calibration errors of 0.5197, 0.7586 and 0.5185, respectively. Fig. 5 shows the fitted training data and calibration by the network with 30 neurons in the hidden layer.
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TABLE 3
SSE values obtained from the networks consists 30 neurons in hidden layer after being trained with 10 000 epochs in different value of learning rate

<table>
<thead>
<tr>
<th>Learning rate</th>
<th>Sum-square error (SSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>120.8410000</td>
</tr>
<tr>
<td>0.0003</td>
<td>2.0346600</td>
</tr>
<tr>
<td>0.0005</td>
<td>1.1228000</td>
</tr>
<tr>
<td>0.0010</td>
<td>0.9130860</td>
</tr>
<tr>
<td>0.0050</td>
<td>0.0199902</td>
</tr>
<tr>
<td>0.0050</td>
<td>0.0714400</td>
</tr>
<tr>
<td>0.0070</td>
<td>6.4872000</td>
</tr>
</tbody>
</table>

Fig. 5: Training data fitting and calibration by the network with 30 neurons in the hidden layer

It was found that, the network with 30 neurons in hidden layer gave the best architecture for generating accurate prediction of V(V) concentration. This network extends the useful response range of the PMMA immobilized FHA in determination of V(V) above 100 mg/L.

Reproducibility Study
Reproducibility refers to the discrepancies in response between individual members of a batch of similar preparation membrane (Yusof and Ahmad, 2002). The results indicate that the developed method is reproducible when used for measurements of V(V) at concentrations of 50 mg/L and 200 mg/L. The relative standard deviations were calculated to be 9.29% and 7.09% for 50 mg/L and 200 mg/L of V(V), respectively. The variation in the determination of V(V) ion using this sensing membrane was due mainly to variation during preparation of the membrane itself which include variation caused by amount of immobilized reagent. Ahmad and Narayanaswamy (2002) reported similar observations in their reproducibility study of the probe in determination of Al(III) ion.
### TABLE 4
The network of V(V) concentration using calibration data

<table>
<thead>
<tr>
<th>Number of neurons in hidden layer</th>
<th>Expected 31</th>
<th>Expected 39</th>
<th>Expected 46</th>
<th>Expected 52</th>
<th>Expected 108</th>
<th>Average calibration errora</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted</td>
<td>Error</td>
<td>Predicted</td>
<td>Error</td>
<td>Predicted</td>
<td>Error</td>
</tr>
<tr>
<td>5</td>
<td>33.5498</td>
<td>2.5498</td>
<td>38.0757</td>
<td>0.9243</td>
<td>42.1415</td>
<td>3.8585</td>
</tr>
<tr>
<td>10</td>
<td>35.8367</td>
<td>4.8367</td>
<td>40.7900</td>
<td>1.7900</td>
<td>45.2066</td>
<td>0.7934</td>
</tr>
<tr>
<td>15</td>
<td>32.3317</td>
<td>1.3317</td>
<td>40.0098</td>
<td>1.0098</td>
<td>46.2469</td>
<td>0.2469</td>
</tr>
<tr>
<td>20</td>
<td>31.7871</td>
<td>0.7871</td>
<td>40.0059</td>
<td>1.0059</td>
<td>46.5330</td>
<td>0.5330</td>
</tr>
<tr>
<td>25</td>
<td>32.8944</td>
<td>1.8944</td>
<td>40.0000</td>
<td>1.0000</td>
<td>46.0542</td>
<td>0.0542</td>
</tr>
<tr>
<td>30</td>
<td>31.7871</td>
<td>0.7871</td>
<td>40.0000</td>
<td>1.0000</td>
<td>46.5330</td>
<td>0.5330</td>
</tr>
<tr>
<td>35</td>
<td>36.9266</td>
<td>5.9266</td>
<td>40.0000</td>
<td>1.0000</td>
<td>46.0349</td>
<td>0.0349</td>
</tr>
<tr>
<td>40</td>
<td>34.0468</td>
<td>3.0468</td>
<td>40.0000</td>
<td>1.0000</td>
<td>44.8721</td>
<td>1.1279</td>
</tr>
</tbody>
</table>

*Average calibration error = \( \sum_{i=1}^{5} | \text{predicted V(V) concentration} - \text{expected V(V) concentration}| / 5 \)
CONCLUSION

ANN trained with Back Propagation (BP) algorithm in the highly non-linear calibration of dynamic range of V(V) was successfully performed in this study. A network architecture consisting of single input neurons, 30 neurons in hidden layer and one output neuron after completing the 10,000 epochs with 0.003% learning rate was found appropriate for the multivariate calibration used.

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