Extending the Linear Dynamic Range of V(V) Ion Determination Based on New Reagent, Fatty Hydroxamic Acid from Palm Kernel Oil by using Artificial Neural Network

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Abstract : An artificial neural network (ANN) has been applied for determination of V(V) by using a new reagent, fatty hydroxamic acid. Spectra obtained from the V(V)-FHA complex at several selected wavelengths are used as the input data for the ANN. The V(V)-FHA complex shows a limited linear dynamic range of V(V) concentration of 0.01 – 250 mg/L. After training with ANN, the linear dynamic range is extended to 1000 mg/L with low calibration error. A three layer feed forward neural network using back-propagation (BP) algorithm has been employed in this study. The input layer consists of five neurons, 38 hidden neurons and one output neuron was found appropriate for the multivariate calibration used. The network were trained up to 50 000 epochs with 0.01 % learning rate. This reagent also provide good analytical performance with reproducibility characters of the method yield relative standard deviation (RSD) of 3.10% and 3.16% for V(V) at concentration of 40 ppm and 260 ppm, respectively. The limit of detection of the method was calculated to be 0.1 ppm.

Keyword: Artificial Neural Network (ANN), V(V), Fatty hydroxamic acid

Introduction

More recently, the application of artificial intelligence or expert systems in analytical chemistry and chemical engineering has emerged rapidly. There are several types of artificial intelligence which had developed such as fuzzy logic, natural language processing and artificial neural network (ANN) [1]. ANN is a computing system made up of a number of simple and highly interconnected processing elements, which processes information by its dynamic state response to external inputs [2].

Rouvray (1993) defined a neural network as a data processing system that simulates the human brain by building on information that it 'learns'. Artificial neurons are much simpler than the biological neuron. The neurons are divided into several layers. The input layer consists of neurons that receive input from the external and the output layer consists of neurons that communicate the output of the system to the external. There are usually a number of hidden layers between these two layers. Each of these inputs is multiplied by a connection weight. In the simplest case, these products are simply summed, fed through a transfer function to generate a result and then output [3].

ANN have been widely used in chemical application such as interpretation of spectra, calibration, process control, molecular dynamics, optimization of the linear signal range, signal processing and modeling structure of protein [4-6]. M-Suah et al has applied ANN on signal processing of optical fibre pH sensor based on bromophenol blue doped with sol-gel film. They successful applied ANN trained with BP algorithm in processing the highly non-linear calibration of an optical fibre pH sensor in their study [7].

The need for V(V) analysis in environmental analysis has increased after a report on different biological role of ionic forms of this species in plants, animals and human. Human exposure to vanadium has severe effects on the cell growth, the cardiac muscle, the diuretic kidney function [8] and the symptoms such as nervous depression, coughing, vomiting, anemia and increased risk of lung cancer, that are sometimes fatal [9]. The neurotoxicity of vanadium can cause somnolence, convulsions,

respiratory failure and gastrointestinal irritation with diarrhea [10].

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 fatty hydroxamic acid. In this study, the fatty hydroxamic acid (FHA) had been synthesized by reacting hydroxylamine with refining, bleached deodorized (RBD) palm kernel olein (liquid phase from the fractionation of palm kernel oil) using lipase as biocatalyst [11]. ANN has been used in quantitative determination of V(V) based on absorption spectrum pattern recognition.

Experimental

Reagent

All chemicals used were of analytical grade and deionized water was used throughout for solution preparation. A stock solution (5.0×10^3 mg/L) of V(V) was prepared by dissolving 0.5 g of V_2O_5 (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 used. A fatty hydroxamic acid (FHA) stock solution of 2.0×10^4 mg/L was prepared by dissolving 20.0021 g of FHA in 1000 mL volumetric flask with methanol.

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 while others at the bottom of the water phases, thus evoking 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.

Procedure

The absorption spectra of 2.0×10^3 mg/L FHA and V(V)-FHA complex were recorded at wavelength 350 to 700 nm. The complex solution was prepared by introducing 3.0 mL of 1.3×10^3 ppm V(V) into a 25 mL volumetric flask containing 9.0 mL of 5.6×10^3 mg/L FHA. The solution was then diluted to the mark with methanol.

The dynamic range study of V(V) determination for linear calibration plot was studied by introducing 3.0 mL of the V(V) solution at different concentration into a volumetric flask containing 9.0 mL of 5.6×10^3 mg/L FHA solution. Then, the volumetric flask was diluted to the mark with methanol. The absorbance was measured at wavelength 455 nm.

The effect of FHA concentration on the V(V) complex formation was studied by adding 3.0 mL of 1.3×10^3 mg/L V(V) solution to a volumetric flask containing $0 - 18$ mL of 5.6×10^3 mg/L FHA (varying amount of FHA). The solution was diluted to the mark with methanol. The absorbance of the complex was obtained at wavelength of 455 nm.

Three different concentrations of analyte were used in the kinetic study of V(V)-FHA complex. The measurements were carried out by introducing 1.0 mL of the V(V) solution at concentration of 40, 100 and 200 mg/L into a cuvette containing 3.0 mL of 2.0×10^3 mg/L FHA. The absorbance was measured at wavelength 455 nm.

The reproducibility study was carried out by measuring the absorbance of ten different batches of similar proportions of mixed solutions. In this study, 3.0 mL of V(V) solution was added into volumetric flask containing 9.0 mL of 5.6×10^3 mg/L FHA. Two different concentrations of V(V) solution were examined in this study, i.e. 40 and 260 mg/L. The absorbance was measured at 455 nm and the relative standard deviation of the measurement was calculated.

The effect of foreign ions on the determination of V(V) were studied by introducing appropriate amount of interfering ions (so that the mole ratio of V(V) : foreign ion is 1:1) into a volumetric flask containing 1.5 mL of 1.3×10^3 mg/L V(V) solution and 9.0 mL of 5.6×10^3 mg/L FHA. The solution was diluted to the mark with methanol. The measurement was made at 455 nm.

The validation study was conducted in this study to compare and validate the data obtained from both ICP-AES and the developed method. A series of V(V) solution at concentrations of 30, 60 and 80 mg/L were prepared. These solutions were analyzed by using both ICP-AES and the method developed in this study. Before analyzing samples by using ICP-AES, a blank and a single standard solution were used to define the calibration curve.

Instrumentation

Spectral measurements were made with an ultraviolet-visible spectrophotometer (Varian-Cary Win UV 100). For each concentration, the spectrum was scanned in the wavelength of $350 - 750$ nm. A total of 20 spectral reading were obtained. Three of these spectra (V(V) concentration of 0.1, 3.0 and 8.0 mg/L) were used for testing the trained network whilst the remaining spectra (1.0 , 4.0, 6.0, 10.0,

16.0, 20.0, 40.0, 60.0, 80.0, 100.0, 180.0, 260.0, 400.0, 640.0, 800.0 and 1000.0 mg/L) were used for the training of 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 five neurons, which represent the absorbance intensities measured at five different wavelengths from each spectrum. The output layer consists of a single neuron which represents the concentration value of V(V). A network having up to 50 neurons in hidden layer has been considered in this study.

The network training and data treatment were realized by using Matlab program [12] 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 50 000 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 its prediction capability and precision.

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.

Table 1 : The general setting of the back-propagation specific parameters during network training

Specific Parameters	Values
Maximum number of epochs to train	50 000
Sum-squared error (SSE) goal	0.02
Learning rate	0.01

Results

Spectral Studies

An aqueous V(V) solution reacts with FHA reagent to form a dark brown complex species. The absorption spectra of the V(V)-FHA complex show a maximum absorbance at wavelength of 455 nm (Figure 1). The reagent alone did not show any significant absorbance. Therefore, a wavelength of 455 nm was chosen for the spectrophotometric measurement of V(V)-FHA complex. Figure 2 shows a three-dimensional (3D) spectra of the V(V-FHA complex at different concentration of V(V) ranging from 0.01 – 1000 mg/L.
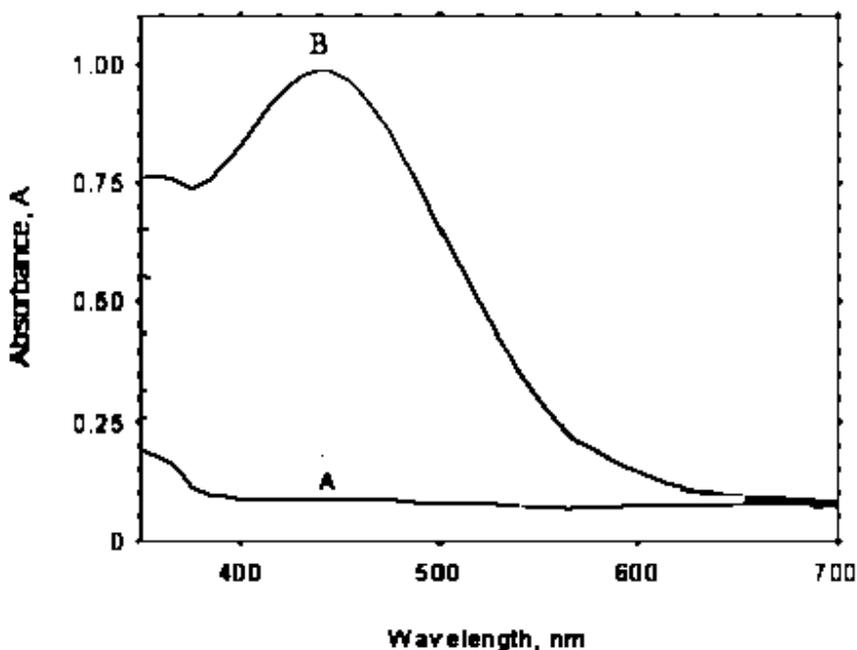

Figure 1 : Absorbance spectra of 2.0 x 10^3 ppm FHA before (A) and after (B) reaction with 150 ppm V(V)
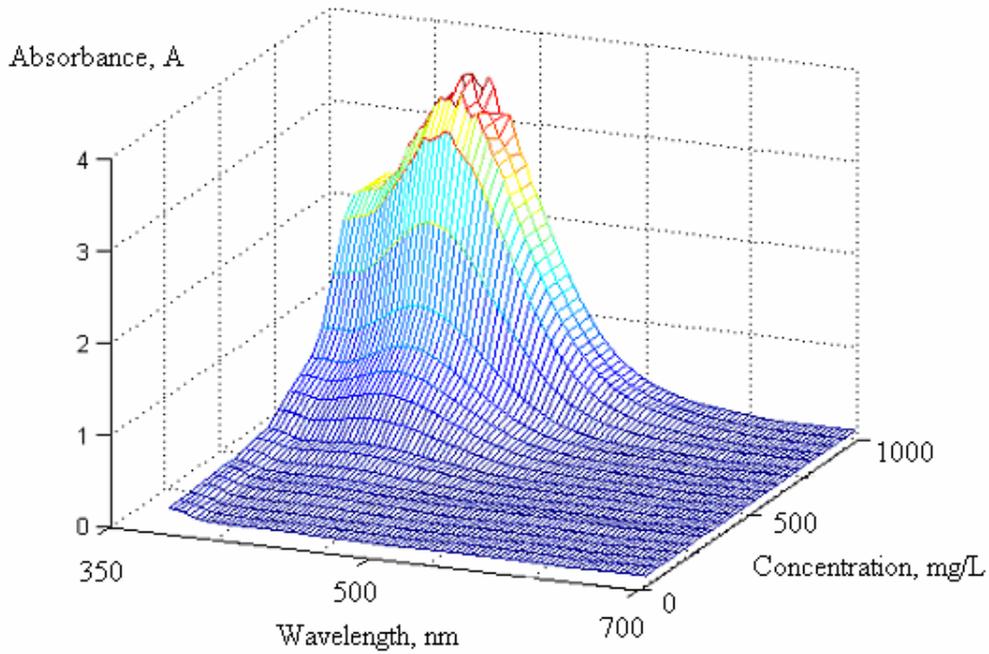
Figure 2 : Generated 3D absorbance spectra for V(V)-FHA complex at different concentration of V(V)

The Dynamic Range of the V(V) Concentration

Figure 3 shows the response curve of the complex towards different concentration of V(V). The method developed produced a linear response when the V(V) concentration is within the range of 1 – 250 mg/L, then almost constant in the range of 400 – 1000 mg/L. The linear part of the response curve is re-plotted as shown in Figure 4. The straight line obtained from this plot can be described by equation $y = 0.0075x + 0.0882$ and the calculated correlation coefficient, r was found to be 0.9881. As shown the beneficial linear range between 0 – 200 mg/L. These kinds of data are suitable for non-linear modeling purposes using ANN.
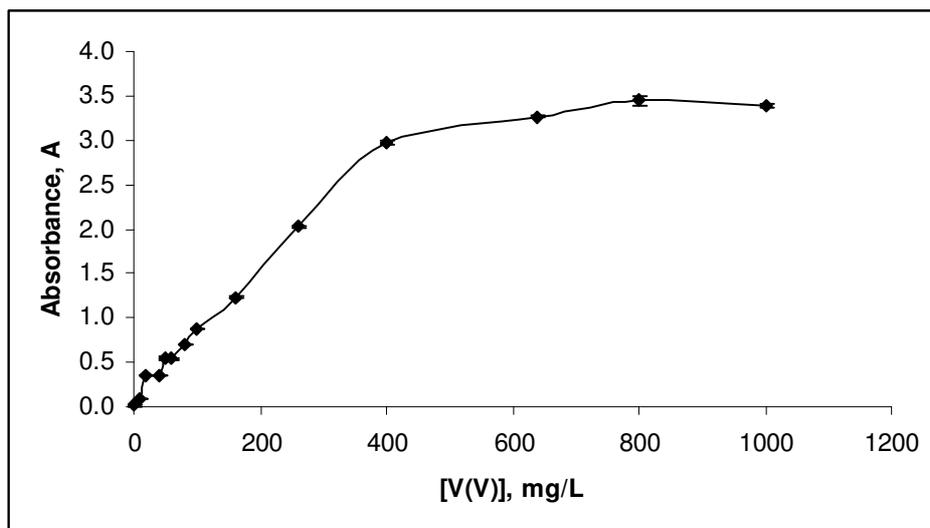

Figure 3 : The response curve of 2.0 x 10³ ppm FHA towards different concentration of V(V)
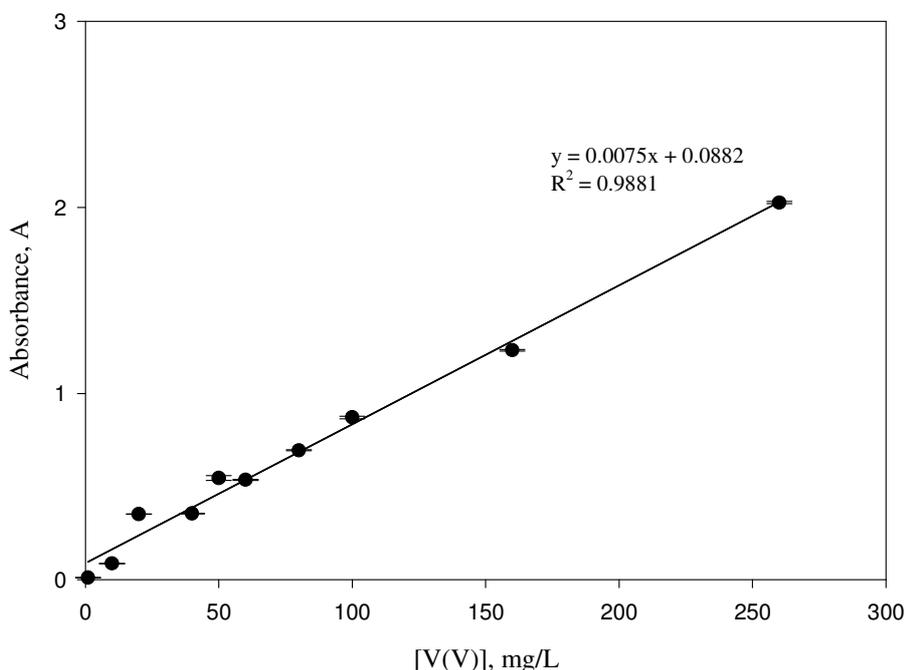
The plot shows Absorbance, A (y-axis, 0 to 3) versus [V(V)], mg/L (x-axis, 0 to 300) with data points and a linear trend line.

$$y = 0.0075x + 0.0882$$
$$R^2 = 0.9881$$

Figure 4 : The linear dynamic range in V(V) determination, where the concentration of FHA was 2.0×10^3 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. By using this definition, the limit of detection of the method was calculated to be 0.1 ppm. The limit of detection of the present developed method was lower compared with previous work as reported by Abbas *et al.* (2001) [13]. They observed that, the limit of detection in determination of V(V) by using first derivative spectrophotometric method was 3.0 ppm.

Multivariate Calibration Using ANN
Data obtained from uv-visible spectrophotometer were used as the input to the ANN. Only five wavelengths points (375, 455, 440, 510 and 625 nm) from each spectrum were chosen to represent the input data for the ANN to avoid several problems during network training periods [14, 15]. All the points selected, were due to their significant variations in the sensor signal.

Sixteen 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, percentage of learning rate. Table 2 shows the SSE values of the network with 20, 25, 30, 35, 38, 41, 45 and 50 hidden neurons after completing the 50 000 epochs.

Table 2 : SSE values obtained from the networks consists of hidden neurons after being trained with 50 000 epochs.

Number of hidden neurons	Sum-square error (SSE) with 50 000 epochs
20	44.6400
25	34.6303
30	13.4456
35	13.5805
38	6.58104
41	10.0236
45	9.26195
50	15.9165

For the network with 20 hidden neurons, the convergence of SSE was observed to be very slow. The fastest convergence of SSE was achieved using 38 neurons in hidden layer. The number of hidden neurons when arranged in declining SSE order was 20, 25, 50, 35, 30, 41, 45, and 38. Network trained with 50 000 epochs were suitable to be used in predicting the response of the concentration of V(V) since it showed a low SSE value. Zupan et al. reported that, ANN training by using much higher number of epochs usually caused problems such as over training and over fitting problems [16]. Three calibration spectra (0.1, 3.0 and 7.0 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 [15, 17].

Table 3 shows the predicted concentration values against the expected concentration values as measured by uv-visible spectrophotometer. As shown, the network with 35, 38 and 41 neurons in hidden layer produced the good predictions results with average calibration errors of 0.4, 0.3 and 0.4, respectively. Figure 5a and Figure 5b shows the training data fitting and calibration by the network with 38 neurons in hidden layer.

Table 3 : The network of V(V) concentration using calibration data

Hidden layer size	Expected 0.1		Expected 3.0		Expected 8.0		Average calibration error[a]
	Predicted	Error	Predicted	Error	Predicted	Error	
20	0.0	0.1	2.9	0.1	6.7	1.3	0.5
25	-0.7	0.8	2.5	0.5	6.7	1.3	0.9
30	-0.3	0.4	2.7	0.3	6.6	1.4	0.7
35	0.3	0.2	3.0	0.0	6.9	1.1	0.4
38	0.1	0.0	3.0	0.0	7.0	1.0	0.3
41	0.3	0.2	3.1	0.1	7.2	0.8	0.4
45	-0.2	0.3	2.8	0.2	6.8	1.2	0.6
50	-0.1	0.2	2.6	0.4	6.5	1.5	0.7

$$^a\text{Average calibration error} = \sum_{i=1}^{3} |\text{predicted V(V) concentration} - \text{expected V(V)}$$
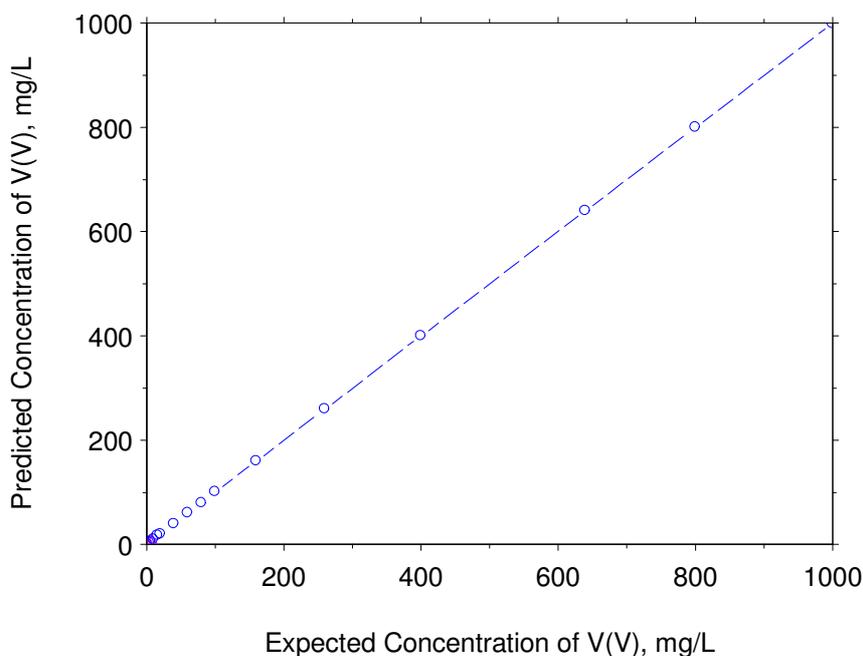
concentration|/3

Figure 5a : Training data fitting and calibration by the network with 38 neurons in hidden layer.
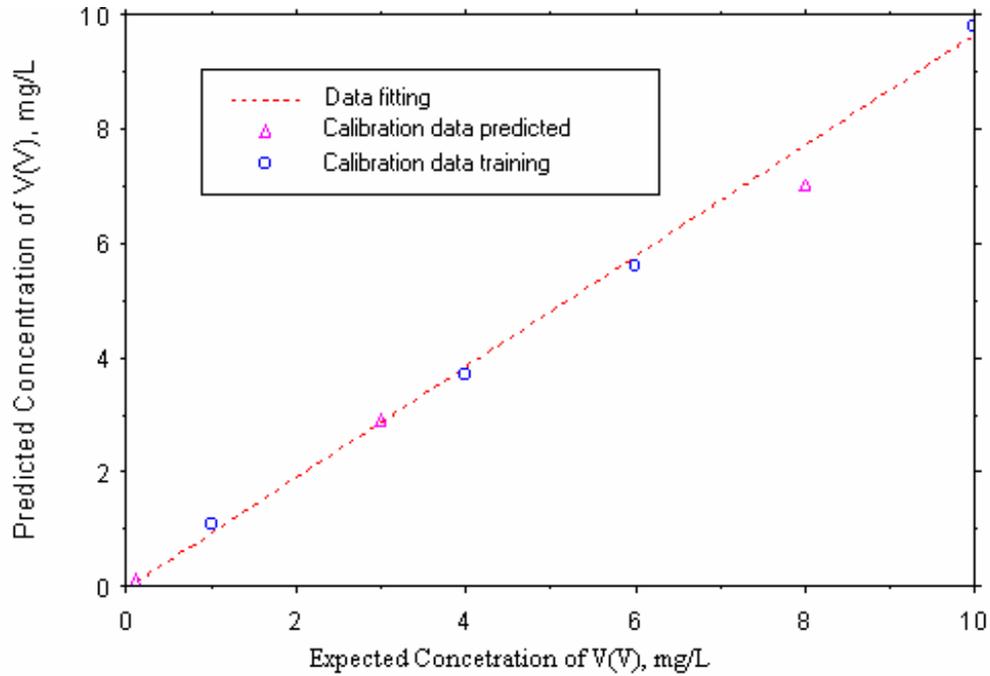
Figure 5b : Training data fitting and calibration by the network with 38 neurons in hidden layer (to show the calibration data point clearly)

It was found that, the network with 38 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 V(V)-FHA complex from V(V) concentration of 1 – 250 mg/L to full calibration range of 400 – 1000 mg/L.
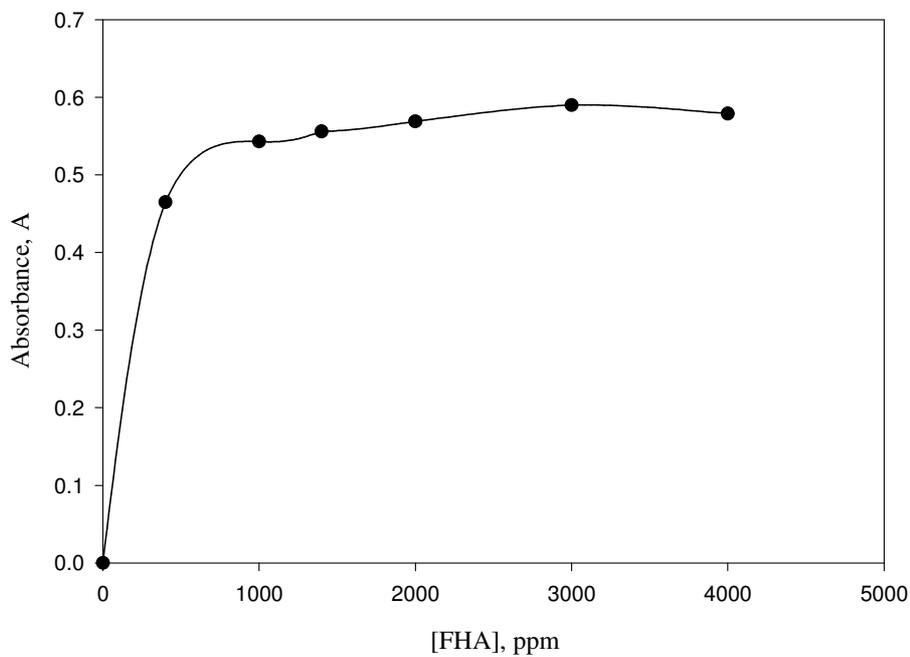

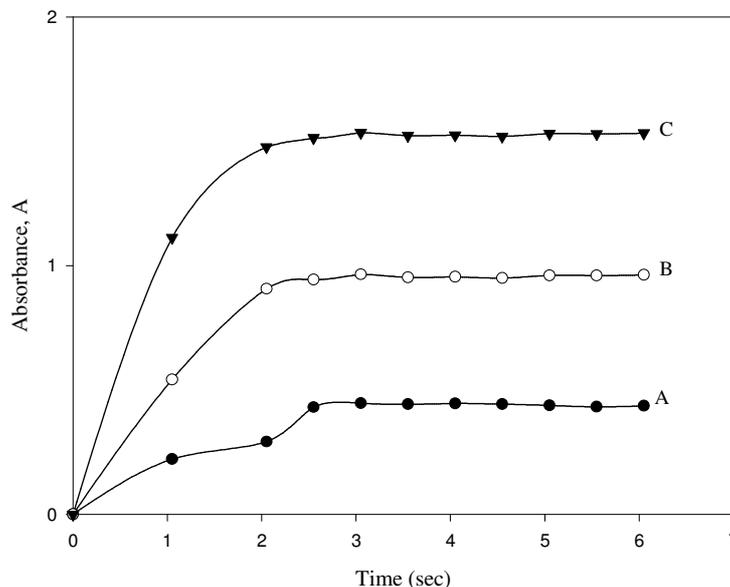**Figure 6 :** Effect of FHA concentration on V(V)-FHA complex formation

Figure 7 :   The kinetic study of V(V)-FHA complex at different concentrations of V(V), i.e. 40 mg/L (A), 100 mg/L (B) and 200 mg/L (C)

The Effect of Amount of FHA

The effect of the reagent concentration on the complex formation (i.e. (V(V)-FHA complex) was studied by using different initial concentration of the FHA $(0 – 4.0 \times 10^3$ mg/L). From Figure 6, it is observed that, the absorbance increased with increasing reagent concentration until it reached a point where the absorbance becomes almost constant. This observation is due to the increasing quantity of the V(V)-FHA complex as the reagent concentration increased. The constant point is reached when all the analyte has been consumed during the complex formation.

The same observation has also been reported by He *et al.* (1999) who studied the spectrophotometric determination of V(V) based on the reaction with 4-(2-pyridylazo)-resorcinol and hydrogen peroxide [18]. The reaction between V(V) and 1,5-diphenylcarbazide which has been studied by Ahmad and Banoo (1999) who also reported the same trend of results [9].

Kinetic Study

Figure 7 shows the kinetic study of V(V)-FHA complex at different concentration of V(V), i.e. 40 ppm, 100 ppm and 200 ppm. The measurements were done in six seconds. It is observed that, the plateau region was achieved after two seconds.

Reproducibility

To determine the applicability and accuracy of this method, a known amount of V(V) concentration was used. The reproducibility study of this proposed method was examined by analyzing two series of ten

solutions having V(V) concentrations of 40 ppm and 260 ppm. The relative standard deviations (RSD) were calculated to be 3.10% and 3.15% for V(V) at concentration of 40 ppm and 260 ppm, respectively. A small RSD values observed for this methods indicate a good precision of the method being used.

Interference Study

In order to asses the usefulness of the proposed method, the effect of foreign ions that often used in industry and presence in factory fluid waste were studied. The effect of foreign ions on determination of V(V) (at 1:1 mole ratio) is summarized in Table 4. The main cation interference was obtained from Fe(III), and this was expected, since the complex formation of Fe(III) with FHA form a dark purple complex. Other ions which also interfered significantly were Zn(II), Cr(III), Cd(II), Co(II), and Cu(II). EDTA and phosphate gave a high degree of interference since they are chemically reactive and capable of forming complex with a lot of metals.

Negative interference was observed for anions whereas cation showed positive interference. The negative interference could result from the reaction of the interfering with the analyte being determined, leading to an incomplete reaction [19]. In some cases anion give positive interference because the interaction of the V(V)-FHA complex with the anion such as citrate and which produce a high absorbance. Ahmad and Narayanaswamy reported that the most common type of such interference is the complexing of the analyte by the interfering ion [20].

Table 4: The degree of interference on complex formation of V(V)-FHA at 1:1 mole ratio of foreign ions:V(V)

Foreign Ions	% Interference
Al(III)	0.7
Cr(III)	3.9
Fe(III)	20.0
Co(II)	2.6
Ni(II)	0.9
Cu(II)	1.4
Zn(II)	5.7
Cd(II)	3.5
ammonium	0.4
citrate	0.7
phosphate	-0.4
EDTA	-0.5

Note: Interference (%) =((x – y) / y) x 100, where x is the average of absorbance value for mixed solution of
V(V) and foreign ions, y is the average of absorbance value for V(V) solution only

Table 5: The validation data of V(V) concentration by the developed method and ICP AES

Concentration of Solution Preparation (ppm)	Developed Method		ICP-AES		t value
	Concentration (mean of three readings)	Standard Deviation	Concentration (mean of three readings)	Standard Deviation	
30.0	35.4	0.077	28.7	0.230	0.729
60.0	59.8	0.336	61.3	0.352	0.038
80.0	81.0	0.278	78.7	0.581	0.543

The t-test is carried out to compare the mean from two set of analysis

Null hypothesis, H_o: $\mu_s = \mu_p$

Alternative hypothesis, H_1: $\mu_s \neq \mu_p$

Where: μ_s= mean from ICP-AES method

μ_p= mean from developed method

$s^2 = \{(n_1-1)s_1^2 + (n_2 -1)s_2^2\}/ (n_1+ n_2-2)$

Where: n_1= number of measurement in ICP-AES method

n_2= number of measurement in developed method

s_1= standard deviation in ICP-AES method

s_2= standard deviation in developed method

$t = \{(|s_1 – s_2|)/ s\} \sqrt{(1/n_1 + 1/n_2)}$

The value of t from 95% confident level with degree of freedom of 4 is 2.78. The calculated t is
less than t from the table. Null hypothesis is accepted. Mean from both method are not
significantly different, indicating that the results from both methods are comparable.

Validation Study

Validation study was conducted to compare and validate the data obtained from the developed method by using an established method of ICP-AES. The results obtained compared favorably with those obtained by ICP-AES as shown in Table 5. The accuracy of the developed method was found to be high as verified by the t-test with multiple samples (paired differences), at a confidence level of 95%, indicating that results obtained by both developed method and ICP-AES are in excellent agreement under identical experimental conditions.

Conclusion

FHA used in this study show a good response in determination of V(V). ANN trained with BP algorithm in the highly non-linear calibration of dynamic range of V(V) has successfully performed in this study. A network architecture consisting of five input neurons, 38 hidden neurons and one output neuron after completing the 50 000 epochs with 0.01 % learning rate was found appropriate for the multivariate calibration used.

Acknowledgements

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